acamprosate was measured in the carcass, suggesting incorporation of the calcium moiety into bone. Protein binding was low in rat, dog, and human plasma, with higher percent binding in rat (overall mean approximately 13.5%) than in dog (2:4%) and human (6%) samples.

Acamprosate pharmacokinetics were studied in mice, rats, rabbits and dogs. Dietary administration of acamprosate at 100 mg/kg/d resulted in Cmax levels below the limit of detection in most mice. Oral acamprosate at 100 mg/kg/d produced peak plasma levels of [redacted] mg/l in rats and [redacted] ng/l in dogs. In comparison, in clinical studies, the proposed 1998 mg/d dose for 8 days resulted in a steady state Cmax of [redacted] mg/l. The increases in Cmax and AUC values were slightly less than dose proportional in the 28-day oral study in rats, with no differences between males and females in the PK parameters. The times to peak plasma concentrations after 100 & 400 mg/kg/d were approximately 0.5 hour in rats and 2 hours in dogs. After 1000 mg/kg/d PO in rabbits, the Cmax of [redacted] mcg eq/ml was detected at 2 hours. The Tmax decreased by approximately 50% with repeated dosing in humans.

The AUC, determined by using the last measurable time point after single dose acamprosate at 400 mg/kg PO, was 44 mg.h/l in rats and 240 mg.h/l in dogs. After 1000 mg/kg/d PO in rabbits, total exposure (AUC) over 24 hours was 522.1 mcg eq.h/ml. In humans the AUC measured from 0-24 hours at steady state after 1998 mg/d for 18 days was 6884 ng.h/l. The half-life of acamprosate after administration of single oral doses of 400 mg/kg was 31 hours in rats and 2.4 hours in beagle dogs. In humans, the half-life at steady state after oral treatment at the dose of 666 mg was 17 hours.

PK/TK conclusions:

Acamprosate bioavailability by the oral route is variable in animals but is generally low. Distribution is primarily to the gastrointestinal tract, kidney and liver, and acamprosate crosses the blood-brain barrier and placenta. There is no evidence of acamprosate metabolism in animals and humans. Oral acamprosate is excreted in feces with a minor fraction excreted in the urine. Protein binding is also low in animals and humans. The PK studies showed peak plasma levels and AUC values increased less than dose proportionally, and there were no differences between males and females in the measured parameters. The Tmax occurred from 0.5-2 hours after oral dosing in rats, rabbits and dogs, and decreased approximately 50% (from approximately 15 hours to 7-9 hours) with repeated dosing.

IV. GENERAL TOXICOLOGY:

Study title: ACAMPROSATE 2-WEEK ORAL TOXICITY STUDY IN MICE. DETERMINATION OF BLOOD LEVELS

Key study findings:

- Mean acamprosate consumption in males 137 and 564 mg/kg/day in the targeted 100 and 400 mg/kg/day dose groups, respectively; in females 125 and 534 mg/kg/day in the targeted 100 and 400 mg/kg/day dose groups, respectively.
- Wounds and hair loss in the high-dose group (534-564 mg/kg/day, dietary) in male and female mice
- Food consumption and body weight gains higher in the male and female mice administered acamprosate compared to controls
- Plasma acamprosate below level of detection (approximately <0.2 mg/l) in 67% of samples at low dose (125-134 mg/kg/d); In the remaining samples the maximum mean plasma levels (across time points) were 0.36 mg/l in the males (range [ ]) and 0.63 mg/l in the females (range [ ].
- Maximum mean levels (across time points) at high dose (534-564 mg/kg/d) were 1.4 mg/l (range [ ] mg/l) in males, and 2.14 mg/l (range [ ] mg/l) in females at 23 and 21 hours respectively.
- Sampling every 2-6 hours demonstrated steady plasma levels over 19 hours.

Study no: 91.05.AOT.001.SP3
Volume # 13, and page #: 1
Conducting laboratory and location: Pharmacokinetic Unit, Lipha Research Centre, 115 Avenue Lacassagne, 69003 Lyon, France
Date of study initiation: March 19, 1991
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot # OTA3011, radiolabel none, and % purity: [ ]
Formulation/vehicle: Admixture with diet, mixed with ground feed

Methods (unique aspects):
Dosing:

Species/strain: [ ] CD-1 VAF (IOPS) mice.
#/sex/group or time point (main study): 12/sex control mice; 72/sex/dose test article
Satellite groups used for toxicokinetics or recovery: None
Age: Not provided
Weight: 29-41 g males, 22-25 g females
Doses in administered units: Target doses 0, 100, 400 mg/kg/d
Route, form, volume, and infusion rate: Oral, by admixture in diet daily for 2 weeks

Observations and times:

Clinical signs: daily
Body weights: weekly

Food consumption: weekly

Clinical chemistry: Blood calcium only: day 15

Toxicokinetics: Plasma acamprosate levels: 1 ml at 7:00 pm, 9:00 pm, 11:00 pm on day 15, 2:00 am, 8:00 am, and 2:00 pm on day 16 (12 different animals/sex/sample time and day)

Results:

Mortality: None

Clinical signs: Wounds and hairloss from muzzle, anterior paw, and dorsal thoracic area in males and females at the high dose

Body weights: Body weight gains in the males mice were 6 g at the low (+100%) and high (+100%) doses compared to a mean gain of 3 g in the controls. In the female mice, body weight gains were 5 g (+67%) at the low dose, and 4 g (+33%) at the high dose, compared to controls (mean gain 3 g).

Food consumption: Mean food consumption was 5.3, 6.0, and 6.0 g/mouse/day in the control, low dose and high dose males, respectively. Mean food consumption was 5.1, 6.1, and 7.3 g/mouse/day in the control, low dose and high dose females, respectively.

Test Article consumption: Mean acamprosate consumption in males was 137 and 564 mg/kg/day in the low and high dose groups, respectively. Mean acamprosate consumption in females was 125 and 534 mg/kg/day in the low and high dose groups, respectively.

Clinical chemistry: No treatment-related effects on blood calcium levels

Toxicokinetics: Below level of detection (approximately <0.2 mg/l) in 67% of samples at the low dose (target 100 mg/kg/d). In the remaining 32% samples at the low dose, the maximum mean plasma levels (across timepoints) were 0.36 mg/l in the males (range [redacted]) and 0.63 mg/l in the females (range [redacted]) Maximum mean plasma levels (across timepoints) at the targeted high dose (400 mg/kg/d) were 1.4 mg/l (range [redacted] mg/l) in males, and 2.14 mg/l (range [redacted] mg/l) in females at 23 and 21 hours respectively. Sampling every 2-6 hours demonstrated steady plasma levels over 19 hours.

Summary of individual study findings: The mean acamprosate intake was 137 and 564 mg/kg/day in the low and high dose (target 100 and 400 mg/kg/day) males, respectively, and 125 and 534 mg/kg/day in the low and high dose (target 100 and 400 mg/kg/day) females, respectively. High dose acamprosate (534-564 mg/kg/day dietary) resulted in wounds and hair loss. Increased food consumption and body weight gains were observed at the low and high dose. Plasma sampling demonstrated that acamprosate was well absorbed by the dietary route in mice at the target levels of 100 and 400 mg/kg/day for 2 weeks. Steady plasma levels were demonstrated when measured periodically over 19 hours, indicating that sampling can be conducted at any time of day.

Study title: AOTA-CA (ACAMPROSATE) SUB ACUTE TOXICITY TO MICE BY DIETARY ADMINISTRATION FOR 13 WEEKS

Key study findings:

- Acamprosate intake comparable to doses intended at 500, 1000, 1500 and 2000 mg/kg/day, dietary
- Increased water consumption in males (18-25%) at 1500 and 2000 mg/kg/day and in females (9-16%) at 1000-2000 mg/kg/day
- Increased urinary calcium and phosphorus in males and females at 2000 mg/kg/day
- Slight decrease in brain, heart, liver, spleen and testes weights in males at doses of 1000-2000 mg/kg/day; decreased brain weights at 1000 and 2000 mg/kg/day and heart weights at 2000 mg/kg/day in females
- MTD was not identified in this study; MTD > 2000 mg/kg/day

Study no: [redacted] 138/88827
Volume # 13, and page #: 82
Conducting laboratory and location: [redacted]
Date of study initiation: November 30, 1987
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot # 1814, radiolabel none, and % purity: [redacted]
Formulation/vehicle: Admixture in diet

Methods (unique aspects):
Dosing:

Species/strain: [redacted] CD-1 mice of Swiss origin
#/sex/group or time point (main study): 10/sex/dose
Satellite groups used for toxicokinetics or recovery: None
Age: 28 days
Weight: Mean 21-24 g males, 18-20 g females
Doses in administered units: 0, 500, 1000, 1500, 2000 mg/kg/d
Route, form, volume, and infusion rate: Oral by admixture in the diet

Observations and times:

Clinical signs: Daily weeks 1-4, then weekly
Body weights: Weekly
Food consumption: Weekly
Water consumption: Daily visually, measured during week 12
Ophthalmoscopy: Not done
EKG: Not done
Hematology: Not done
Clinical chemistry: Serum calcium, phosphorus only, week 13
Urinalysis: Urine calcium, phosphorus, volume only, week 13
Gross pathology: Week 13: Superficial tissues, brain, pituitary gland, cranial nerves, all subcutaneous tissues, thoracic viscera (particularly thymus, lymph nodes, heart), abdominal

viscera, urinary bladder, gastro-intestinal tract (including stomach, cecum), lungs, liver, kidneys, gonads, adrenals, uterus, intra-abdominal lymph nodes, accessory reproductive organs

Organs weighed: Week 13; adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid, uterus

Histopathology: Week13; heart [control and HD groups] and kidneys [all groups]; all other organs and macroscopically abnormal tissues were preserved for future examination if required

Toxicokinetics: Not done

Other: None

Results:

Mortality: None

Clinical signs: No treatment-related effects

Body weights: No treatment-related effects

Food consumption: No treatment-related effects

Acamprosate Intake: 508, 993, 1514 and 2036 mg/kg/d in males, and 511, 1016, 1513 and 2040 mg/kg/d in females in groups designated 500, 1000, 1500 and 2000 mg/kg/d, respectively, over weeks 1-13.

Water consumption: Treatment related increase in males at 1500 (52 g/mouse/day, +18%) and 2000 (55 g/mouse/day, +25%) mg/kg/day, and in females at 1000 (47 g/mouse/day, +9%) 1500 (50 g/mouse/day, +16%), and 2000 (49 g/mouse/day, +14%) mg/kg/day compared to control water consumption (44 and 43 g/mouse/day in males and females, respectively).

Ophthalmoscopy: Not done

Electrocardiography: Not done

Hematology: Not done

Clinical chemistry: Increased plasma calcium (2%) in females at 1000, 1500 and 2000 mg/kg/d; No change in plasma phosphorus.

Urinalysis: Increased urinary calcium in males (+260%) and females (+200%) at 2000 mg/kg/day; Increased urinary phosphorus in males (+17%) and females (+60%) at 2000 mg/kg/day.

Organ weights: The treatment-related effects in organ weights are presented in the following table:

Organ Weight Changes in Mice Given Dietary Acamprosate for 13 Weeks

Organ	0 mg/kg/day	500 mg/kg/day	1000 mg/kg/day	1500 mg/kg/day	2000 mg/kg/day
Males					
Brain (g)	0.5	0.5	0.4 (-20%)	0.4 (-20%)	0.4 (-20%)
Heart (g)	0.20	0.20	0.20	0.19 (-5%)	0.18 (-10%)
Liver (g)	2.09	2.30	1.60** (-23%)	1.66** (-21%)	1.83* (-12%)
Spleen (g)	0.090	0.109	0.089 (-1%)	0.073 (-19%)	0.083 (-8%)
Testes (g)	0.35	0.35	0.35	0.34 (-3%)	0.30** (-14%)
Females					
Brain (g)	0.5	0.5	0.4	0.5	0.4

			(-20%)		(-20%)
Heart (g)	0.16	0.16	0.16	0.16	0.12** (-25%)

*p<0.05; **p<0.01

Gross pathology: No treatment-related effects
Histopathology: No treatment-related effects
Toxicokinetics: Not done

Summary of individual study findings: Acamprosate intake was demonstrated at levels comparable to those intended at 500, 1000, 1500 and 2000 mg/kg/day. Water consumption was increased in males by 18-25% at 1500 and 2000 mg/kg/day, and in females by 9-16% at doses from 1000-2000 mg/kg/day. Increased urinary calcium and phosphorus were observed in males and females at 2000 mg/kg/day. Brain, heart, liver, spleen and testes weights were slightly decreased in males at doses of 1000-2000 mg/kg/day; and brain weights were decreased at 1000 and 2000 mg/kg/day and heart weights decreased at 2000 mg/kg/day in females. The MTD was not established in this study. No toxicity that would be expected to have an impact on survival was observed. This is in agreement with the sponsor's conclusion that acamprosate at up to 2000 mg/kg/day by dietary administration showed no dose-limiting toxicity, because the increase in water consumption and urinary calcium excretion were not associated with histopathological changes in the kidney.

Study title: ACAMPROSATE 3-WEEK ORAL TOXICITY STUDY IN RATS. DETERMINATION OF BLOOD LEVELS

Key study findings:

- Dose related increase in body weight gain in females by 100% and 229% at 100 and 400 mg/kg/day, respectively, compared to controls. Body weight gains decreased in the males 41% and 4% at 100 and 400 mg/kg/day, respectively, compared to controls.
- Food consumption decreased in males 8% and 2% at 100 and 400 mg/kg/day, respectively, and increased 8% at 100 and 400 mg/kg/day in females compared to controls.
- Plasma acamprosate not detected in rats at 100 mg/kg/day; mean concentrations in males 0.98-3.33 mg/l and in females 0.75-4.29 mg/l at 400 mg/kg/day sampled at 2-6 hour intervals on treatment days 22 and 23
- Dietary acamprosate adequately absorbed at the high dose, indicated by plasma concentrations; peak plasma levels in the morning; no differences between males and females in plasma levels

Study no: 91.07.AOT.001.RP4
Volume # 13, and page #: 266
Conducting laboratory and location: Pharmacokinetic Unit, Lipha Research Centre, 115 Avenue Lacassagne, 69003 Lyon, France
Date of study initiation: June **4, 1991**
GLP compliance: Yes
QA report: yes (x) no ( )

Drug, lot # OTA3011 , radiolabel none, and % purity: [ ]
Formulation/vehicle: Admixture in diet

Methods (unique aspects):
Dosing:

Species/strain: CD-VAF (IOPS) Sprague-Dawley rats [ ]
#/sex/group or time point (main study): 18/sex/dose
Satellite groups used for toxicokinetics or recovery: None
Age: Not provided
Weight: 410-560 g males, 328-535 g females
Doses in administered units: Target doses: 0, 100 and 400 mg/kg/day
Actual doses received: Males: 0, 137, 564 mg/kg/day; Females: 0, 125, 534 mg/kg/day
Route, form, volume, and infusion rate: Oral by admixture in the diet, daily for 21 days

Observations and times:

Clinical signs: Daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Not done
EKG: Not done
Hematology: Not done
Clinical chemistry: 1 ml blood withdrawn from 3 animals/group/timepoint at 7:00, 9:00, 11:00 pm on Day 22; 2:00 and 8:00 am and 2:00 pm on day 23; calcium levels measured only
Urinalysis: Not done
Gross pathology: Not done
Organs weighed: Not done
Histopathology: Not done
Toxicokinetics: 1 ml blood withdrawn from 3 animals/group/timepoint at 7:00, 9:00, 11:00 pm on Day 22; 2:00 and 8:00 am and 2:00 pm on day 23,
Other: None

Results:

Mortality: None
Clinical signs: No treatment-related signs
Body weights: Dose related increase in body weight gains in females: +100% and +229% at 100 and 400 mg/kg/day, respectively, compared to controls.
Body weight gains decreased in males at 100 mg/kg/day (-41%) and 400 mg/kg/day (-4%) compared to controls.
Food consumption: Decreased in males 8% and 2% at 100 and 400 mg/kg/day, respectively, and increased 8% at 100 and 400 mg/kg/day in females compared to controls.
Ophthalmoscopy: Not done
Electrocardiography: Not done
Hematology: Not done
Clinical chemistry: No significant treatment-related changes in calcium
Urinalysis: No treatment-related changes
Organ weights: Not done

Gross pathology: Not done

Histopathology: Not done

Toxicokinetics: Plasma acamprosate undetected (limit of detection 0.2 mg/l) in the rats that received 100 mg/kg/day. Mean plasma acamprosate levels (mg/l) in the rats that were administered 400 mg/kg/day dietary acamprosate are presented in the following table:

Plasma Acamprosate (mg/l) in Rats Administered 400 mg/kg/day Dietary

Sampling Time	Males	Females
7 pm	1.7	0.75
9 pm	0.98	0.75
11 pm	1.22	3.02
2 am	1.6	2.54
8 am	2.28	4.29
2 pm	3.33	2.65

Summary of individual study findings: There was a dose related increase in body weight gain in females (100% and 229% at 100 and 400 mg/kg/day, respectively) compared to controls, and a decrease in males (41% and 4% at 100 and 400 mg/kg/day, respectively). Food consumption decreased slightly in the males (8% and 2% at 100 and 400 mg/kg/day, respectively), and increased 8% at 100 and 400 mg/kg/day in females compared to controls. Plasma acamprosate was not detected in rats at 100 mg/kg/day; mean plasma concentrations in the males were 0.98-3.33 mg/l and in the females were 0.75-4.29 mg/l at 400 mg/kg/day, sampled at 2-6 hour intervals on treatment days 22 and 23. The results of this study showed that dietary acamprosate was adequately absorbed at the high dose, although the lower limit of detection (0.2 mg/l) precluded detection at the low dose. Peak plasma levels occurred in the morning, with no differences between males and females.

Study title: AOTA-CA (ACAMPROSATE) SUB ACUTE TOXICITY TO RATS BY DIETARY ADMINISTRATION FOR 13 WEEKS

Key study findings:

- Loose feces in males and females: sporadic at 500 mg/kg/day (weeks 8 and 11) and 1000 mg/kg/day (weeks 7-13), more frequently observed at 2000 mg/kg/day (weeks 7-13)
- Increased water consumption in males at 1000 and 2000 mg/kg/day and females at 2000 mg/kg/day
- Decreased urinary volume in females at 500 (31%), 1000 (34%), and 2000 (37%) mg/kg/day; Increased urinary Ca in males at 1000 (183%) and 2000 (1200%) mg/kg/day, and females at 1000 (173%) and 2000 (540%) mg/kg/day
- Increased adrenal weights in males at 2000 mg/kg/day (11%); Decreased ovarian (14%) and heart (7%) weights in females at 2000 mg/kg/day
- Treatment-related firm contents in ileum, watery distension and pale contents in cecum, and soft, pale colon contents in males at 500 mg/kg/day, and males and females at 1000 and 2000 mg/kg/day
- Target organs of toxicity included GI and urinary systems; not all organ systems were assessed.
- MTD identified as 1000 mg/kg/day, dietary based upon renal effects

Study no: [redacted] 139/88834
Volume # 14, and page # 1
Conducting laboratory and location: [redacted]
Date of study initiation: December 1, 1987
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot # 1814, **radiolabel:** none, **and % purity:** [redacted]
Formulation/vehicle: Powdered drug substance mixed into dietary feed, homogeneity confirmed by chemical analysis of feed samples in weeks 1 and 13

Methods (unique aspects):
Dosing:
Species/strain: [redacted] CD rats of Sprague-Dawley origin
#/sex/group or time point (main study): 10/sex/dose
Satellite groups used for toxicokinetics or recovery: None
Age: 28 days
Weight: 122-123 g males and 98-99 g females
Doses in administered units: 0, 500, 1000 & 2000 mg/kg/day; target doses achieved based on analysis of test diets
Route, form, volume, and infusion rate: Oral by admixture in diet

Observations and times:
Clinical signs: Daily weeks 1-4, then weekly
Body weights: Weekly
Food consumption: Weekly
Water consumption: Week 12
Ophthalmoscopy: Not done
EKG: Not done
Hematology: Not done
Clinical chemistry: Calcium and Inorganic phosphorus only, Week 13
Urinalysis: Volume, urinary calcium and inorganic phosphorus only, Week 13
Gross pathology: Week 13
Organs weighed: Week 13: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid, uterus
Histopathology: heart (control and HD) and kidneys (all groups) only, remaining organs preserved for future examination, Week 13
Toxicokinetics: Not done
Other: None

Results:
Mortality: None
Clinical signs: The incidence of loose feces was increased at the highest dose from weeks 7 onward, and was more prominent in males. The incidence of loose feces is presented in the following table:

Incidence of Loose Feces in Rats Administered Dietary Acamprosate

Dose (mg/kg/d)	Cage Number	Treatment Week* 1	2	3	4	5	6	7	8	9	10	11	12	13
Males														
0	1											1		
	2													
500	3													
	4													
1000	5								1		1	1	3	1
	6													
2000	7					1		2	3	5	3	3	5	5
	8							2	4	6	3	3	5	5
Females														
0	9													
	10													
500	11								1					
	12											1		
1000	13												2	
	14													
2000	15								2	1	3		1	2
	16								1	1		2		1

*Values represent #days during the treatment week that loose feces observed in each cage.

Soiled urogenital region was observed at 1000 mg/kg/day from week 12-13 in 10% males and in week 11 in 3% females, and at 2000 mg/kg/day from week 9-13 in 100% males and 10%-21% females.

Body weights/body weight gains: No statistically significant treatment-related effects

Food consumption: No treatment-related effects

Acamprosate Intake: 497, 993 & 2005 mg/kg/d in males; 498, 997 & 1998 mg/kg/d in females in the groups designated 500, 1000 & 2000 mg/kg/d respectively, averaged over weeks 1-13.

Water consumption: Significantly increased in male rats at 1000 mg/kg/day (283±24.3 g/rat/day, +30%) and 2000 mg/kg/day (335±24.7 g/rat/day, +53%) compared to control males (219±5.2 g/rat/day), increased in females at 2000 mg/kg/day (253±17.5 g/rat/day, +20%) compared to control females (210±17.1 g/rat/day).

Ophthalmoscopy: Not done

Electrocardiography: Not done

Hematology: Not done

Clinical chemistry: Presented in the table below

Urinalysis: Urinary volume was decreased by 31-37% in females and urinary calcium was increased by 2.8x-13x in the mid-dose and high-dose males and 2.7x-6.4x in the females. The results of the urinalysis are presented in the table below:

Results of Urinalysis and Clinical Chemistry Evaluation in Rats Administered Dietary Acamprosate

	Biochemistry		Urinalysis		
Dose (mg/kg/d)	Ca (mEq/L)	P (mEq/L)	Volume (ml)	Ca (mcEq/vol)	P (mcEq/vol)
Males					
0	5.6	3.6	4.6	12	552
500	5.5	3.8	3.8	15	534
1000	5.6	4.0**(11%)	4.0	34**(183%)	510
2000	5.9** (5%)	4.0**(11%)	3.6	155**(1200%)	524
Females					
0	5.4	3.3	3.2	30	461
500	5.6* (4%)	3.3	2.2*(31%)	33	374
1000	5.5*(2%)	3.2	2.1**(34%)	82**(173%)	447
2000	5.9**(9%)	3.5	2.0**(37%)	192**(540%)	340

*p<0.05; **p<0.01

Organ weights: Decreased liver weight in males at 1000 & 2000 mg/kg/d (19% and 17%, respectively). Increased adrenal weights in males at 2000 mg/kg/d (11%). Decreased ovarian & heart weights in females at 2000 mg/kg/d (14% and 7%, respectively).

Gross pathology: The results of the gross observations are presented in the following table (values represent incidence):

Observation	Males Dose (mg/kg/d)				Females Dose (mg/kg/d)			
	0	500	1000	2000	0	500	1000	2000
Ileum: contents firm	0	1	2	9	0	1	0	4
Caecum: watery distension	0	1	2	10	0	0	0	5
Contents pale	0	9	8	10	0	0	7	10
Colon: contents soft	0	6	4	10	0	0	3	10
Contents pale	0	9	9	10	0	0	5	10

Histopathology: No treatment-related effects on heart and kidneys

Toxicokinetics: Not done

Summary of individual study findings: Dietary administration of acamprosate in male and female rats for 13 weeks resulted in loose feces in the males and females at 1000 and 2000 mg/kg/day from weeks 7-13, and in 1 female at 500 mg/kg/day during weeks 8 and 11. The incidence of loose feces was sporadic at the low-dose and mid-dose, and was more frequent at the high dose. Increased water consumption was observed in males at 1000 and 2000 mg/kg/day and females at 2000 mg/kg/day. The urinalysis showed decreased urinary volume in females at 500 (31%), 1000 (34%), and 2000 (37%) mg/kg/day, increased urinary Ca in males at 1000 (183%) and 2000 (1200%) mg/kg/day, and females at 1000 (173%) and 2000 (540%) mg/kg/day. Decreased liver weights were found in males at 1000 (19%) and 2000 (17%) mg/kg/day, and increased adrenal weights in males at 2000 mg/kg/day (11%). Ovarian (14%) and heart (7%) weights were decreased in females at 2000 mg/kg/day. Treatment-related firm contents in the ileum, watery distension and pale contents in the cecum, and soft, pale colon contents were observed in males at 500 mg/kg/day, and males and females at 1000 and 2000 mg/kg/day. The

MTD in this study was 1000 mg/kg/day based upon renal effects. The sponsor concluded that no major signs of toxicity were observed up to 2000 mg/kg/day, and the treatment-related findings (loose feces, increased water consumption, increased plasma and urinary calcium, and minor changes in gastrointestinal pathology) were observed primarily at 2000 mg/kg/day and without histopathological changes in the kidney.

Study title: THREE MONTH REPEAT DOSE ORAL TOXICITY STUDY OF CALCIUM ACETYLHOMOTAURINATE (ACAMPROSATE IN RATS FOLLOWED BY A THIRTY DAY REVERSIBILITY PERIOD

Key study findings:

- Salivation and liquid diarrhea in 1 high dose rat
- Increased adrenal weights in males at 960 (30%) and 2400 (27%) mg/kg/day
- Distended kidney tubule sections from coagulum accumulations, early senile nephrosis in 2 high dose male and 1 high dose female recovery rats
- Target organs of toxicity adrenals, kidneys
- MTD >2400 mg/kg/day
- NOAEL was not identified due to lack of full histopathology evaluation

Study no: 1097
Volume # 14, and page # 155
Conducting laboratory and location: [redacted]
Date of study initiation: Report date given only: December 9, 1983
GLP compliance: No
QA report: yes (x) no ( )
Drug, lot # 1395/3, **radiolabel:** none, **and % purity:** not provided
Formulation/vehicle: drug substance dissolved in sterile distilled water

Methods (unique aspects):
Dosing:

Species/strain: OFA Sprague-Dawley rats [redacted]
#/sex/group or time point (main study): 9/sex/dose
Satellite groups used for toxicokinetics or recovery: 6/sex at 2400 mg/kg/day reversibility group
Age: 32 days
Weight: 100 ± 20 g males and females
Doses in administered units: 0, 320, 960, and 2400 mg/kg/day, daily for 90 days
Route, form, volume, and infusion rate: Oral intubation by metal cannula at 1 ml/100 g bodyweight

Observations and times:

Clinical signs: Daily
Body weights: 2x weekly
Food consumption: 2x weekly

Ophthalmoscopy: cornea, conjunctive, iris, retina, days 0 and 90

EKG: Not done

Hematology: Days 0, 45 and 90 in treatment groups, 120 in reversibility group

Clinical chemistry: Days 0, 45 and 90 in treatment groups, 120 in reversibility group

Urinalysis: Treatment days 25-28, 80-83

Gross pathology: Day 90 in control, treatment groups, day 120 in reversibility group

Organs weighed: liver, kidneys, heart, spleen adrenals, gonads

Histopathology: stomach, small and large intestine, liver, kidneys, heart, spleen, adrenals, thyroid, gonads (full histopathology assessment was not performed)

Toxicokinetics: Not done

Other: None

Results:

Mortality: Deaths in 1 male and 1 female at 320 mg/kg/day, and 1 male at 960 mg/kg/day due to intubation errors (confirmed by gross examination)

Clinical signs: No individual line listings provided for the clinical signs; Sponsor reported "temporary" salivation and liquid diarrhea in 1 high-dose rat (sex, timing, frequency not provided)

Body weights: No treatment-related effects

Food consumption: No treatment-related effects

Ophthalmoscopy: No treatment-related effects

Electrocardiography: Not done

Hematology: No treatment-related effects

Clinical chemistry: No treatment-related effects

Urinalysis: No treatment-related effects

Organ weights: Increased liver weight at 320 mg/kg/day but not at the higher doses in males and females (25% and 11% respectively), gonad weight at 2400 mg/kg/day in males (7%) and adrenals at 960 and 2400 mg/kg/day in males (30% and 27%, respectively); no difference from controls in the reversibility groups. No correlating histopathology findings were observed.

Gross pathology: No treatment-related effects

Histopathology: Distended kidney tubule sections from coagulum accumulations attributed to early senile nephrosis in 3 high dose recovery animals (2 males, 1 female)

Toxicokinetics: Not done

Summary of individual study findings: Acamprosate administration by daily oral intubation for 90 days in rats resulted in salivation and liquid diarrhea in 1 high-dose animal (no information on sex, time of observation, frequency provided). Increased gonad weight (7%) was observed at 2400 mg/kg/day, and increased adrenal weight was observed at 960 (30%) and 2400 (27%) mg/kg/day in the male rats; these changes were reversible. The histopathology showed distended kidney tubule sections from coagulum accumulations attributed to early senile nephrosis in 3 high dose recovery rats (2 males and 1 female). The MTD was considered to be >2400 mg/kg/day, in agreement with the sponsor. A NOAEL can not be identified because a full histopathology assessment was not conducted.

Study title: AOTA-Ca (ACAMPROSATE) - 4 WEEK INTRAVENOUS TOXICITY STUDY IN THE BEAGLE DOG

Key study findings:

- IV doses in beagle dogs (20, 100 and 200 mg/kg/day) represent 0.25x-2.7x the MRHD of 1998 mg/d in a 50 kg patient on a BSA basis
- Dose-related increase in incidence of vomiting and injection site swelling and induration,
- Occasional treatment-related salivation, tremors, chewing, and agitation observed
- Slight increase in plasma calcium and decrease in phosphorus
- Treatment-related induration, swelling, hemorrhagic infiltration, periphlebitis and granulomatous inflammation at the injection site
- Lower plasma levels in the females compared to the males from 1 hour to 4 hours after dosing, suggesting higher clearance of acamprosate in the females
- NOAEL was not observed due to vomiting and injection site effects in a small number of low-dose dogs. No systemic toxicity was identified.

Study no: 35191 ([ ] study number 439/021)
Volume # 18, and page #: 1
Conducting laboratory and location: [ ]
Date of study initiation: 21 August, 1991
GLP compliance: Yes
QA report: yes (x) no ( )
Drug Acamprosate, **lot #** 00166, **radiolabel** none, **and % purity:** [ ]
Formulation/vehicle: Test article in water for injection, sterilized by filtration [ ]

Methods (unique aspects):
Dosing:

Species/strain: Beagle dogs [ ]
#/sex/group or time point (main study): 3/sex/dose group
Satellite groups used for toxicokinetics or recovery: None
Age: 5 months
Weight: 5.9-9.5 kg males, 5.3-7.8 kg females
Doses in administered units: 0, 20, 100, 200 mg/kg/day (high dose based on MTD determined in study 439/020)
Route, form, volume, and infusion rate: Intravenous (cephalic or external saphenous vein) at 25, 100 and 200 mg/ml (1 ml/kg/day) over 1 minute, once daily for 28 days in males and 29 days in females
Control Article: saline for injection (NaCl 0.9%)

Observations and times:

Clinical signs: 2x daily
Body weights: 1x weekly
Food consumption: 1x daily
Ophthalmoscopy: Baseline and week 4

EKG: Baseline, day 2 and week 4 before and 30 minutes after dosing: diastolic and systolic blood pressure, heart rate, rhythm and cardiac conduction

Hematology: Baseline and week 4

Clinical chemistry: **Baseline and week 4**

Urinalysis: Baseline and week 4 (16 hours per collection day)

Gross pathology: Day 29 (males) or 30 (females): external surface, all orifices, cranial cavity, external surface of brain and samples of spinal cord, thoracic, abdominal and pelvic cavities and viscera, cervical tissues and organs, carcass, injection sites

Organs weighed: Day 29 (males) or 30 (females); See Histopathology Table below

Histopathology: Day 29 (males) or 30 (females); See Histopathology Table below

Toxicokinetics: Blood drawn (jugular vein, 5 ml) before dosing and at 30 min, and 1, 2, and 4 hours after dosing on dosing days 1 and 28

Other: None

Results:

Mortality: No deaths

Clinical signs: Vomiting in 3 or 4 low and mid-dose dogs (total of 3 and 5 occasions, respectively), in all high dose dogs (total of 58 occasions in 6 dogs, days 4,5,8,18) within 30 minutes of dosing; occasional salivation, tremors, chewing, agitation in mid- and high dose dogs; dose-related increase in incidence swelling and induration at injection site

Body weights: No treatment-related effects

Food consumption: No treatment-related effects

Ophthalmoscopy: No treatment-related effects

Electrocardiography: No treatment-related effects

Hematology: No treatment-related effects

Clinical chemistry: Slight increase in calcium and slight decrease in phosphorus at the high dose compared to controls

Urinalysis: No treatment-related effects

Organ weights: No treatment-related effects

Gross pathology: Treatment-related induration, swelling and hemorrhagic infiltration at the injection site

Macroscopic Observations (values represent incidence)

	Males (dose in mg/kg/d)				Females (dose in mg/kg/d)			
	0	20	100	200	0	20	100	200
Injection Site Number Examined	3	3	3	3	3	3	3	3
Induration	0	2	1	2	0	0	1	2
Swelling	2	2	1	2	0	1	2	2
Hemorrhagic Infiltration	2	1	1	1	1	2	1	2
Necrosis	0	0	0	0	0	1	0	0

Histopathology: Dose related increase in incidence and severity of periphlebitis with granulomatous inflammation at the injection site

Microscopic Observations (values represent incidence)

	Males (dose in mg/kg/d)				Females (dose in mg/kg/d)			
	0	20	100	200	0	20	100	200
Injection Site Number Examined	3	3	3	3	3	3	3	3
Periphlebitis	0	0	1	3	0	1	1	3
Granulomatous inflammation	0	2	1	3	0	1	2	3

Toxicokinetics:

Mean Plasma Acamprosate (ng/ml)

		20 mg/kg/day IV		100 mg/kg/day IV		200 mg/kg/day IV	
		Males	Females	Males	Females	Males	Females
Day 1	Before Dosing	0	0	0	0	0	0
	0.5h	36007	33558	136761	139518	402854	314554
	1h	20545	21193	107993	95222	333020	351639
	2h	4956	3784	30837	28855	101004	89320
	4h	692	396	2755	1596	6790	4551
Day 28	Before Dosing	<LOD*	<LOD*	<LOD*	<LOD*	145	118
	0.5h	38352	24862	259371	359149	445996	441547
	1h	12373	11714	265400	284379	206235	198753
	2h	3978	3201	60866	75494	56863	45225
	4h	496	292	2024	2620	4731	3913

*Limit of detection 5 ng/ml

Summary of individual study findings: Intravenous treatment with acamprosate at doses of 20, 100 and 200 mg/kg/day in beagle dogs resulted in vomiting and injection site swelling and induration, with a dose-related increase in incidence. Treatment-related salivation, tremors, chewing, and agitation were observed occasionally. In the clinical chemistry evaluation, there was a slight increase in calcium and decrease in phosphorus levels. The macroscopic and microscopic examinations at necropsy showed treatment-related induration, swelling, hemorrhagic infiltration, periphlebitis and granulomatous inflammation at the injection site. The plasma acamprosate measurements showed lower plasma levels in the females compared to the males from 1 hour to 4 hours after dosing, suggesting higher clearance of acamprosate in the females. A NOAEL was not observed due to vomiting and injection site effects in a small number of low-dose dogs. No definitive systemic toxicity was observed.

Study title: PRELIMINARY 4-WEEK ORAL TOXICITY STUDY IN THE DOG

Key study findings:

- Liquid diarrhea in all animals at 1000 mg/kg/day
- Reversible decreases in body weights (5%-7%) in the male dogs
- A NOAEL was not identified.

Study no: 408231
Volume # 17, and page #: 1
Conducting laboratory and location: [redacted]
Date of study initiation: July 10, 1984
GLP compliance: Yes

QA report: yes (x) no ( )
Drug Acamprosate, **lot #** 1395/11, **radiolabel** None, **and % purity:** Not provided in the Certificate of Analysis
Formulation/vehicle: Test article dissolved in distilled water

APPEARS THIS WAY
ON ORIGINAL

Methods (unique aspects):
Dosing:

Species/strain: Beagle dogs [redacted]
#/sex/group or time point (main study): 2/sex
Satellite groups used for toxicokinetics or recovery: Not used
Age: Not provided
Weight: 7.5-10.5 kg
Doses in administered units: 1000 mg/kg/day
Route, form, volume, and infusion rate: Oral by gastric intubation at 5 ml (20% solution), daily for 29 days

Observations and times:

Clinical signs: Daily
Body weights: Baseline and 2x weekly
Food consumption: Baseline and daily
Ophthalmoscopy: Baseline and end of study
EKG: Not done
Hematology: Baseline and end of study
Clinical chemistry: Baseline and end of study
Urinalysis: Not done
Gross pathology: Day 30
Organs weighed: Day 30: Liver, kidneys, spleen, adrenals, gonads
Histopathology: Not done
Toxicokinetics: Not done
Other: None

Results:

Mortality: No deaths
Clinical signs: Intermittent liquid diarrhea in all animals after each drug administration
Body weights: Slight decrease in body weights (approximately 5%-7% lower than baseline values) in the males in the first 2 weeks, reversed during the subsequent two weeks of treatment
Food consumption: No treatment-related effects
Ophthalmoscopy: No treatment-related effects
Electrocardiography: Not done
Hematology: No treatment-related effects
Clinical chemistry: No treatment-related effects
Urinalysis: Not done
Organ weights: No conclusions can be made because no controls were used for comparison
Gross pathology: No treatment-related effects
Histopathology: Not done
Toxicokinetics: Not done

Summary of individual study findings: A NOAEL was not identified in this study due to liquid diarrhea in all animals after dosing and lack of full systemic evaluation.

Study title: SEVEN DAY SUBACUTE TOXICITY STUDY IN THE MACAQUE MONKEY BY ORAL ADMINISTRATION OF CALCIUM ACETYLHOMOTAURINATE (ACAMPROSATE) (Study Summary provided only, Study report published in French)

Key study findings:

- Oral acamprosate administered by gavage at 1 g/kg/day in cynomolgus monkeys represented approximately 8x the MRHD of 1998 mg/d in a 50 kg patient on a BSA basis
- 7 days treatment resulted in liquid diarrhea in all animals throughout dosing
- Slight decrease in body weights (-5% to –6%), probably due to liquid diarrhea
- NOAEL cannot be determined due to lack of clinical pathology and histopathologic examination, lack of a control group and limited numbers of animals.

Study no: 1605
Volume # 18, and page #: 303
Conducting laboratory and location: [redacted]
Date of study initiation: November 13, 1986
GLP compliance: Yes
QA report: yes (x) no ( )
Drug Acamprosate, **lot #** 1395/11, **radiolabel** None, **and % purity:** Not reported
Formulation/vehicle: Test article dissolved in distilled water

Methods (unique aspects):
Dosing:

Species/strain: Macaque monkey , [redacted]
#/sex/group or time point (main study): 2 males and 1 female
Satellite groups used for toxicokinetics or recovery: None
Age: Not provided
Weight: 3.25-3.55 kg males, 2.35 kg female
Doses in administered units: 1 g/kg/day
Route, form, volume, and infusion rate: Oral gavage, 20% solution , 5 ml/animal

Observations and times:

Clinical signs: Daily
Body weights: 2x weekly
Food consumption: Daily
Ophthalmoscopy: Not done
EKG: Not done
Hematology: Not done
Clinical chemistry: Not done

Urinalysis: Not done

Gross pathology: Day 8: thoracic and abdominal cavities and corresponding viscera
Organs weighed: Not done
Histopathology: Not done
Toxicokinetics: Not done
Other: None

Results:

Mortality: No deaths
Clinical signs: Liquid diarrhea was observed in all 3 animals throughout treatment
Body weights: Slight reduction in body weights

Body Weights in Cynomolgus Monkeys Given Oral Acamprosate at 1 g/kg/d for 7 Days

Animal Number	Weight on Day 1	Weight on Day 8
Male 2611	3.25 kg	3.10 kg (-5%)
Male 2612	3.55 kg	3.35 kg (-6%)
Female 2613	2.35 kg	2.20 kg (-6%)

Food consumption: No treatment-related effects
Ophthalmoscopy: Not done
Electrocardiography: Not done
Hematology: Not done
Clinical chemistry: Not done
Urinalysis: Not done
Organ weights: Not done
Gross pathology: No treatment-related effects
Histopathology: Not done
Toxicokinetics: Not done

Summary of individual study findings: Oral acamprosate administration (gavage) at 1 g/kg/day (approximately 8x the MRHD of 1998 mg/d in a 50 kg patient on a BSA basis) for 7 consecutive days in cynomolgus monkeys resulted in liquid diarrhea in all animals throughout the dosing period. The slight decrease in body weights (-5% to –6%) was probably a result of the liquid diarrhea, as no change in activity and food consumption were noted. The NOAEL cannot be determined because clinical pathology and histopathologic examination were not performed, limited numbers of animals were evaluated and no control group was included.

Study title: AOTA-CA (ACAMPROSATE) TWENTY-SIX WEEK ORAL TOXICITY STUDY IN THE RAT FOLLOWED BY A SIX WEEK REVERSIBILITY PERIOD

Key study findings:

- Deaths at 2400 mg/kg/d in male and female rats
- Treatment-related clinical signs piloerection, subdued behavior, hypothermia, sudden weight loss in animals that died; dose-related ptyalism, soft feces in rats that survived to sacrifice
- Dose-related increase in water consumption

- Slight treatment-related increases in alkaline phosphatase in females, blood urea nitrogen in males and females, serum and urinary calcium in males and females, acidified urine in males and females
- Dose-related decrease in heart weight in males and females, spleen in males; dose-related increase in adrenal and kidney weights in males and females
- Treatment-related gross pathology observations in the stomach, cecum, ileum, and colon (distension, liquid contents, gas, hypertrophy) in males and females
- Target organs of toxicity included the kidneys, GI (stomach, ileum, cecum, colon), heart, adrenals, spleen, pancreas and brain
- A NOAEL dose was not identified; an increased incidence of stomach findings and cerebellar vacuolation was noted at the mid-dose, and low dose animals were not evaluated.

Study no: 602201.1986
Volume # 15, and page #: 1
Conducting laboratory and location: [redacted]

Date of study initiation: October 1, 1984
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot # 1395/11, radiolabel: none, **% purity** [redacted]
Formulation/vehicle: White crystalline powder drug substance dissolved in distilled water

Methods (unique aspects):
Dosing:
Species/strain: OFA Sprague-Dawley rats [redacted]
#/sex/group or time point (main study): See under Allocation of Animals
Satellite groups used for recovery: See under Allocation of Animals

Allocation of Animals

Group	Group Designation	Week of Animal Sacrifice			
		26 (treatment animals)		32 (6-wk recovery animals)	
		Males (n)	Females (n)	Males (n)	Females (n)
1	Control	20	20	10	10
2	Low Dose	15	15	5	5
3	Mid-Dose	15	15	5	5
4	High-Dose	30	30	-	-

Age: 5-6 weeks
Weight: 123-131 g males, 131-135 g females
Doses in administered units: 0, 320, 960, 2400 mg/kg/day
Route, form, volume, and infusion rate: Oral by gastric intubation at 10 ml/kg

Observations and times:
Clinical signs: Daily
Body weights: Weekly
Food consumption: Weekly

Water consumption: Weeks 1, 4, 9, 13, 18, 22, 26, 27, 31
Ophthalmoscopy: Baseline, weeks 13, 26
EKG: Not done
Hematology: Baseline, weeks 4, 13, 26, 32
Clinical chemistry: baseline, weeks 4, 13, 26, 32
Urinalysis: Baseline, weeks 4, 13, 26, 32
Gross pathology: 26 weeks & 32 weeks (reversibility group)
Organs weighed: 26 weeks & 32 weeks (reversibility group): brain, pituitary, thyroid and parathyroid, heart, liver, spleen, kidneys, adrenals, gonads
Histopathology: 26 weeks & 32 weeks (reversibility group): thoracic aorta, abdominal aorta, brain (cerebellum, stem), caecum, heart, colon, duodenum, epididymides, stomach, liver, submaxillary lymph node, mesenteric lymph node, mammary gland, submaxillary salivary gland, adrenals, pituitary, ileum, jejunum, spinal cord (cervical, lumbar), voluntary muscle, sciatic nerve, eye, esophagus, ovaries, bone (femur), pancreas, skin, lungs, prostate, spleen, rectum, kidneys, testes, thymus, thyroid, trachea, uterus, seminal vesicles, urinary bladder, treatment-related macroscopic abnormalities, bone marrow smear. The low dose group was not evaluated.
Toxicokinetics: Not done
Other: None

Results:

Mortality: Deaths in the low dose (320 mg/kg/day, 1 female on day 92) and mid-dose groups (960 mg/kg/day, 1 male and 1 female) were attributed to intubation errors. In the high dose group (2400 mg/kg/day), 3 males and 2 females were found dead after intubation errors, and 5 males (16.7%) & 17 females (56.7%) died between the weeks of 15 and 26 with evidence of microscopic renal lesions

Clinical signs: Clinical signs in the animals that died in the high-dose group (treatment-related deaths) were piloerection, subdued behavior, hypothermia, sudden weight loss, remaining animals showed intubation errors. In the surviving animals, dose-related ptyalism, soft feces (liquid diarrhea in the high dose) observed as described in the following table:

Clinical Signs in Rats Administered Oral Acamprosate Daily for 26 Weeks

Clinical Sign	0 mg/kg/day n=30	320 mg/kg/day n=20	960 mg/kg/day n=20	2400 mg/kg/day n=30
Ptyalism		5M,2F (wk 8-26)	18M,10F (wk 6-26)	30M,30F (wk 6-26)
Soft Feces		20M (wk12-26)	20M, 20F (wk 6-26)	30M 30F (wk1-26)
Convulsions				1F (d 78,89,122)
Subcutaneous palpable masses (mammary adenoma or adenocarcinoma)		1F (wk15-26)		1F (wk 16-26) 1F (wk 22-26)

Body weights: No treatment-related effects
Food consumption: No treatment-related effects
Water Consumption: Dose-related increase in all treated groups, presented in the following table:

Percent Change in Water Consumption Compared to Controls

Acamprosate Dose	Week 1	Week 4	Week 9	Week 13	Week 18	Week 22	Week 26
Males:							
320 mg/kg/day	0	+4	+1	+3	+5	-6	+10
960 mg/kg/day	+11	+10	+5	+22	+32	+31	+37
2400 mg/kg/day	+16	+35	+39	+53	+71	+77	+74
Females:							
320 mg/kg/day	-2	-3	0	+6	+17	+16	+16
960 mg/kg/day	0	+5	+9	+22	+28	+26	+29
2400 mg/kg/day	+24	+27	+38	+67	+88	+88	+72

Ophthalmoscopy: No treatment-related effects
Electrocardiography: Not done
Hematology: No treatment-related effects
Clinical chemistry: See under Clinical Pathology table below
Urinalysis: See under Clinical Pathology table below

Results of the Clinical Pathology Evaluation (week 26)

Observation	0 mg/kg/day n=30	320 mg/kg/day n=20	960 mg/kg/day n=20	2400 mg/kg/day n=30
Biochemistry				
Alkaline phosphatase (mu/ml)	M: 44±9 F: 19±6	M: 43±13 (-2%) F: 22±9 (16%)	M: 35±7 (-21%) F: 23±10 (21%)	M: 45±7 (2%) F: 27±7 (42%)
Blood urea nitrogen (g/l)	M: 0.37±0.03 F: 0.39±0.03	M: 0.41±0.04 (11%) F: 0.40±0.05 (3%)	M: 0.41±0.04 (11%) F: 0.44±0.06 (13%)	M: 0.47±0.06 (27%) F: 0.44±0.06 (13%)
Calcium (mg/l)	M: 103±2 F: 104±4	M: 108±4 (5%) F: 106±3 (2%)	M: 110±3 (7%) F: 111±2 (7%)	M:118±6 (15%) F: 117±6 (13%)
Urinalysis				
Volume (ml/16h)	M: 10.1±2.9 F: 8.2±3.9	M: 7.6±2.8 F: 7.2±6.5	M: 5.9±2.0 F: 3.6±2.6	M: 13.1±3.4 F: 6.2±3.4
Calcium (mg/l)	M: 127±56 F: 304±159	M: 322±107 (153%) F: 654±404 (115%)	M: 760±287 (498%) F: 1716±660 (464%)	M: 2602±352 (1948%) F: 2305±742 (658%)

Urine was slightly acidified in 3/10 LD females, 4/10 MD males and 7/10 MD females (HD urine not tested due to presence of fecal matter), measured in week 26. There was a dose-related increase in urinary proteins, hemoglobin and pincushion crystals in the male and female urine. The increase in urinary calcium was not proportional to dose.

No treatment-related abnormalities in biochemistry and urinalysis were observed after the 6-week reversibility period.

Organ weights: At 26 weeks, significantly decreased heart and spleen weights were observed in the HD males, increased adrenal weights in HD males and MD and HD females, and

increased kidney weights in the HD males and females. No effects were noted in the reversibility groups at 32 weeks. The treatment-related organ weight changes at 26 weeks are presented in the following table:

Treatment-Related Organ Weight Changes

Organ		0 mg/kg/day	320 mg/kg/day	960 mg/kg/day	2400 mg/kg/day
Heart (g)	Males	1.703±0.139	1.697±0.152 (-1%)	1.613±0.158 (-5%)	1.526± 0.129 (-10%)
	Females	1.107±0.099	1.043±0.082 (-6%)	1.021±0.063 (-8%)	1.033±0.089 (-7%)
Spleen (g)	Males	0.855±0.139	0.788±0.108 (-8%)	0.809±0.113 (-5%)	0.499±0.075 (-42%)
Adrenal (mg)	Males	53±8	56±5 (+6%)	58±8 (+9%)	64±10 (+21%)
	Females	52±9	59±8 (+13%)	63±8 (+21%)	64±14 (+23%)
Kidneys (g)	Males	3.225±0.361	3.330±0.377 (+3%)	3.049±0.227 (-5%)	3.597±0.501 (+11%)
	Females	1.986±0.207	1.857±0.254 (-6%)	1.901±0.161 (-4%)	2.135±0.257 (+8%)

Treatment-Related Changes in Relative (to body weight) Organ Weights

Organ		0 mg/kg/day	320 mg/kg/day	960 mg/kg/day	2400 mg/kg/day
Heart (g)	Males	0.305±0.03	0.289±0.022 (-5%)	0.288±0.020 (-6%)	0.283±0.018 (-7%)
	Females	0.330±0.027	0.336±0.024 (+2%)	0.324±0.024 (-2%)	0.332±0.021 (+1%)
Spleen (g)	Males	0.152±0.019	0.135±0.019 (-11%)	0.144±0.016 (-5%)	0.137±0.020 (-10%)
	Females	0.168±0.03	0.179±0.048 (+7%)	0.158±0.022 (-6%)	0.154±0.022 (-8%)
Kidneys (g)	Males	0.577±0.066	0.566±0.047 (-2%)	0.546±0.039 (-5%)	0.666±0.078 (+15%)
	Females	0.592±0.057	0.599±0.079 (+1%)	0.601±0.043 (+1%)	0.687±0.089 (+16%)
Adrenal (mg)	Males	9±2	10±1 (+11%)	10±2 (+11%)	12±2 (+33%)
	Females	17±3	19±2 (+12%)	20±2 (+18%)	21±4 (+24%)

Gross pathology: The gross observations showed treatment-related effects in the stomach, cecum, ileum, and colon. The results of the gross pathology evaluation are presented in the following table:

Results of the Gross Pathology Evaluation (Week 26)

Observation	Control (n=20/sex)	320 mg/kg/d (n=15M, 14F)	960 mg/kg/d (n=14/sex)	2400 mg/kg/d (n=25 M, 13F)
Thymic Lymph Node: Hypertrophy	3F	1M	3M, 2F	7M
Congestion	3F			2M
Stomach: Distension				3M, 1F
Liquid contents				2M, 1F
Blood			1F	
Ileum: Gas		1M, 1F		4M
Distension	2M	2M		4M, 1F
Caecum: Hypertrophy		1M	2M, 1F	15M, 5F
Gas		2M	1M, 1F	5M, 5F
Distension	2M	2M	5M, 10 F	19M,12 F
Liquid Contents	1M	2M	6M, 9F	19 M, 9F
Colon: Hypertrophy			1M	1F
Gas		2F	1F	2M, 4F
Distension	1M	2M, 1F	2M, 10F	9M, 9F
Liquid Contents	1M	2M	3M, 9F	13M, 8F
Uterus: Hypertrophy	2F	3F	1F	5F
Nodule		1F	1F	
Tumor-like Mass			1F	
Hydrometric Appearance	2F	3F	1F	5F
Subcutaneous; Tumor-like Mass				1F

Gross abnormalities in the reversibility animals (n=1-/sex) included moderate to marked involution of the thymus in 5 males and 6 females, distension of the jejunum, ileum, cecum and colon in 1 female, uterine hypertrophy and hydrometric appearance in 1 female, dysmorphosis of the spleen in 1 female, and hypertrophy of the thyroid in 1 female. In the low-dose reversibility animals (n=5/sex), the observations were lumpy liver in 1 male, involution of the thymus in 1 male and 4 females, hemorrhagic nodule in the uterine horn in 2 females, endometrial malpighian metaplasia in 1 female, and gaseous distension in the stomach in 1 female. In the mid-dose reversibility animals (n=5/sex), the observations were gaseous distension in the cecum in 1 male, involution of the thymus in 2 males and 3 females, and hypertrophy and hydrometric appearance in the uterine horn in 2 females. Gross observations were not performed in high-dose reversibility animals.

The gross findings in the animals that died during the study included advanced autolysis of the digestive tract, lung inflammation, acute inflammation and multiple adhesions in the esophagus, pericarditis in the heart, dry pleurisy of the lung, advanced thymic involution and acute thymus inflammation, hypoplasia of the white pulp (spleen), acute salivary gland inflammation and edema, adhesions of the trachea and thymus to adjacent organs, intestinal gas, hemorrhagic eye and thoracic cavity, mottled liver, dyschromia and whitish spots in the kidney, intracellular and extracellular mineralization in the kidney, wall of the abdominal and thoracic aorta, lung, stomach, duodenum, ileum and cecum, liquid contents in the stomach and intestine, cardiac induration and dyschromia, liver and kidney pallor, and kidney hydronephrosis.

Histopathology: In the 22 high-dose animals that died in week 15: 2/5 males & 13/17 females had renal lesions. The results of the histopathology examinations on animals sacrificed or found dead during the treatment period are presented in the following table:

Results of the Microscopic Examination in Animals that Died or Were Sacrificed During the Treatment Period

Observation	Control (n=0/sex)	320 mg/kg/d (n= 1F)	960 mg/kg/d (n=1/sex)	2400 mg/kg/d (n=3-4 M, 14-16F)
Liver: Vacuolation				1M
Congestion				2F
Lymphohistiocytic Infiltrate				1F
Kidney: Vacuolation				2M, 10F
Lithiasis (calculi)				1M, 2F
Tubular Ectasia				2M, 5F
Renal Pelvic Distension				2F
Intracellular Mineralization				4F
Epithelial Atrophy				1M, 1F
Bladder: Lithiasis				1F
Heart: Extracellular Mineralization				6F
Myolysis				5F
Myocarditis		1F		1F
Pericarditis		1F		
Abdom. Aorta: Mineralization				1F
Lung: Foam Cells				2F
Edema				4F
Lymphohistiocytic infiltrate				1M
Hemorrhagic				1F
Mineralized Focus				1F
Thymus: Involution		1F		1M, 4F
Spleen: Hypotrophy				3F
Stomach:Extracellular mineralization				2M, 12F
Hyperkeratosis				1M, 2F
Dyskeratosis				5F
Polymorphic Inflammatory Infiltrate				1M, 1F
Fibrosis				2F
Duodenum: Extracel. Mineralization				3F
Interstitial Edema				1F
Focal Necrosis				1F
Caecum: Interstitial Edema				3F
Polymorphic Inflammatory Infiltrate				3F

The results of the histopathology examinations on animals sacrificed at the end of the treatment period are presented in the following table. Low dose animals were not evaluated.

Results of the Microscopic Examination of Animals Sacrificed at the End of Treatment

Observation	Control (n=20/sex)	320 mg/kg/d (n=0 evaluated)	960 mg/kg/d (n=14/sex)	2400 mg/kg/d (n=25 M, 13F)
Liver: Vacuolation	7F		4F	2M, 5F
Chronic Inflammation			1F	2F
Lymphohistiocytic Infiltrate	2M			1M
Fibrosis				1M
Kidney: Vacuolation	4 M		1M	3M, 1F
Tubular Casts	1M, 1F		4M	2M1F
Lithiasis (calculi)				6M, 3F
Tubular Ectasia	1M			5M, 3F
Renal Pelvic Distension			1M	
Intracellular Mineralization				3M, 1F
Epithelial Atrophy				2M, 2F
Bladder: Lymphohistiocytic Infiltrate				1M
Polymorphic Inflam. Infiltrate				2M
Ectasia				1M
Heart: Extracellular Mineralization				1F
Fibrosis				2M
Myocarditis				7M, 1F
Lymphohistiocytic Infiltrate	1M		3M	3M
Abdom. Aorta: Mineralization				1M
Lung: Foam Cells	2M, 2F		1M, 1F	2M, 2F
Edema				1M
Lymphohistiocytic infiltrate	15M, 18F		10M, 7F	22M, 10F
Microcrystals				1M
Mineralized Focus				3M
Brain, Cerebellum: Thrombus				3M
Vacuolation			1M, 3F	2M, 5F
Thymic Lymph Node: Hyperplasia	2F		3M	6M, 2F
Stomach:Extracellular mineralization				23M, 9F
Hyperkeratosis				10M, 5F
Dyskeratosis	5M, 2F		9M, 3F	23 M, 9F
Polymorphic Inflammatory Infiltrate			2M	5M, 6F
Duodenum: Extracel. Mineralization				6M
Caecum: Interstitial Edema				1M
Thinning Organ Wall				2M, 1F
Colon: Ectasia			1F	11M, 7F
Adrenal: Vacuolation			1F	1M, 1F
Pancreas: Vacuolation	7M, 8F		9M, 5F	13M, 3F

The results showed dose-related renal tubulopathy in 10/27 interpretable females and 5/29 males, with foamy degeneration of tubular epithelial cells, mineralization, lithiasis from weeks 15 (females) or 16 (males) on; renal, cardiac, digestive & vascular tissue calcifications; cardiac myocarditis, myolysis in females that died; hyperkeratosis & dysplasia of stomach. In addition, vacuolation of the cerebellum was observed in mid- and high-dose animals (low dose not assessed) and thrombus was noted at the high dose. The subcutaneous palpable masses were found to be mammary adenocarcinoma in one low-dose female and mammary adenomas in 2 high-dose females, and were not considered to be treatment-related because these are commonly found in this species.

In the reversibility animals, endometrial malpighian metaplasia was observed in 1 low-dose female, and pancreatic vacuolation in 1 mid-dose female. Microscopic examination was not performed on the high-dose reversibility animals.

Toxicokinetics: Not done

Summary of individual study findings: Drug related deaths occurred at 2400 mg/kg/d in male (17%) and female (57%) rats between the weeks of 15 and 26. Treatment-related clinical signs in these animals included piloerection, subdued behavior, hypothermia, and sudden weight loss. Dose-related ptyalism and soft feces were observed in the rats that survived to sacrifice. In the clinical chemistry, increases in alkaline phosphatase were observed in the females, blood urea nitrogen in males and females, and serum and urinary calcium in males and females, with acidified urine in the males and females. The necropsy showed dose-related decreases in heart weight in males and females and spleen in males, and dose-related increases in adrenal and kidney weights in both males and females. Gross pathology observations were predominantly in the gastrointestinal system, including distension, liquid contents, gas, hypertrophy. The histopathology in the animals that died showed liver and kidney vacuolation, kidney lithiasis, tubular ectasia, renal pelvic distension, heart mineralization, myolysis, and myocarditis, abdominal aortic mineralization, lung foam cells, edema, lymphohistiocytic infiltrate, hemorrhage and mineralized focus, thymus involution, spleen hypertrophy, stomach extracellular mineralization, hyperkeratosis and dyskeratosis with polymorphic inflammatory infiltrate and fibrosis, duodenum extracellular mineralization, interstitial edema and focal necrosis, and cecum interstitial edema and polymorphic inflammatory infiltrate. The treatment-related histopathology observations in the surviving animals were in kidney, bladder, heart, lung, brain, thymus, stomach and small and large intestines, and adrenal; the abnormalities included tubular casts, lithiasis, ectasia, renal pelvic distension, intracellular mineralization and epithelial atrophy; extracellular mineralization, fibrosis, myocarditis; and lymphohistiocytic infiltrate in bladder, heart, lung, stomach.. The NOAEL was not identified in this study due to an increased incidence of dyskeratosis and inflammation of the stomach and vacuolation of the cerebellum at the mid-dose and the lack of histopathology evaluation at the low dose. The sponsor's conclusion was that doses of 320-960 mg/kg/day were well tolerated but 2400 mg/kg/day produced severe metabolic disorders, soft tissue calcifications and cardiac, stomach and renal lesions

Study title: AOTA-Ca (ACAMPROSATE) TWENTY-SIX WEEK ORAL TOXICITY STUDY IN THE BEAGLE DOG

Key study findings:

- Diarrhea in all treated dogs, with a dose-related increase in incidence and severity
- Cardiac rhythm and conduction abnormalities possibly related to acamprosate administration including several observations of 1st degree and 2nd degree auriculo-ventricular heart block, ventricular premature beat at the high dose
- Serum potassium slightly decreased and chloride was slightly increased in the high dose male dogs
- Dose-related increase in urinary calcium in all acamprosate-treated animals
- NOAEL not established due to diarrhea and increased urinary calcium at the low dose

- No definitive target organs of toxicity were identified.
- Doses studied represent 3.4X to 13.5X the MRHD

Study no: 509215
Volume # 17, and page #: 34
Conducting laboratory and location: [redacted]
Date of study initiation: October 23, 1984
GLP compliance: Yes
QA report: yes (x) no ( )
Drug Acamprosate, **lot #** 1395/11, **radiolabel** None, **and % purity:** Not provided in the Certificates of Analysis
Formulation/vehicle: Test article dissolved in distilled water

Methods (unique aspects):
Dosing:

Species/strain: Beagle dogs [redacted]
#/sex/group or time point (main study): 4/sex/dose
Satellite groups used for toxicokinetics or recovery: None
Age: 6 months
Weight: Approximately 7.3-8.7 kg
Doses in administered units: 0, 250, 500, 1000 mg/kg/day, daily for 26 weeks
Route, form, volume, and infusion rate: Oral by gastric intubation at 5 ml/kg

Observations and times:

Clinical signs: Daily before and after treatment
Body weights: Baseline and weekly
Food consumption: Baseline and daily
Water consumption: Baseline and weeks 1, 5, 13, and 25
Ophthalmoscopy: Baseline and weeks 13 and 26
EKG: Baseline, and before and 90 minutes after treatment on Day 1, weeks 13 and 26
Hematology: Baseline and weeks 4, 13, and 26
Clinical chemistry: Baseline and weeks 4, 13, and 26
Urinalysis: By catheterization at baseline (all animals) and weeks 4 (control and high dose), 13 (control and high dose), and 26 (all animals)
Gross pathology: Day after last administration (Day 184)
Organs weighed: Day after last administration (Day 184); see under Histopathology Inventory below
Histopathology: Day after last administration (Day 184); see under Histopathology Inventory below
Toxicokinetics: Not done
Other: None

Results:

Mortality: No deaths
Clinical signs: Diarrhea was observed in all treated dogs as follows: sporadic throughout

treatment period at the low dose, after each acamprosate administration at the mid- and high dose with greater severity after the high dose, beginning several hours after each administration

Body weights: No treatment-related effects

Food consumption: No treatment-related effects

Water consumption: No treatment-related effects

Ophthalmoscopy: No treatment-related effects

Electrocardiography: No treatment-related effects on blood pressure or heart rate or QT interval. Cardiac rhythm and conduction abnormalities, possibly related to acamprosate administration, were observed in 1 control female (1 2nd degree auriculo-ventricular heart block at baseline), 2 high dose males (1 2nd degree auriculo-ventricular heart block 90 min after first dose plus 1 1st degree block before administration in week 13 in one dog, 1 ventricular premature beat at lead II before administration in week 13 in the second dog) and 1 high dose female (several 2nd degree auriculo-ventricular blocks before administration in week 13). No treatment related effects at the low and mid doses, nor in week 26.

Hematology: No treatment-related effects compared to baseline and controls.

Clinical chemistry: No treatment-related effects compared to baseline and controls in weeks 4 and 13; Slight decrease in potassium and slight increase in chloride in male dogs at week 26 (without dose relationship, within historical limits), probably not treatment related

Results of Potassium and Chloride Measurements in Male Dogs

Group/Animal number	Baseline	Week 4	Week 13	Week 26
Potassium (mEq/l)				
Control Dog 151	4.8			4.8
152	4.4			
153	4.7	4.7		4.7
154	4.6			
Low Dose Dog 159	4.1			
160	4.2			
161	4.2			
162	4.3			4.3
Mid-Dose Dog 167	4.8			
168	4.6			
169	4.6			
170	4.1			4.1
High Dose Dog 175	4.4	4.4		
176	4.2			
177	4.5	4.5		
178	5.0	5.0		
Chloride (mEq/l)				
Control Dog 151	110			
152	107			
153	109			
154	111			
Low Dose Dog 159	112			
160	108		108	
161	110		110	
162	108		108	
Mid-Dose Dog 167	111			
168	108			
169	108			
170	110			

High Dose Dog 175	110		110	
176	107	107		
177	108			
178	108			

Urinalysis: A dose-related increase in calcium was noted, which is likely related to excretion of test substance.

Mean Urinary Calcium (Week 26, mg/l, ±SD)

	0 mg/kg/day	250 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
Male Dogs	46 ± 23	108 ± 82 (X2.3)	172 ± 25 (X3.7)	481 ± 247 (X10.5)
Female Dogs	28 ± 13	161 ± 37 (X5.75)	162 ± 84 (X5.75)	295 ± 97 (X10.5)

Organ weights: No treatment-related effects
Gross pathology: No treatment-related effects
Histopathology: No treatment-related effects
Toxicokinetics: Not done

Summary of individual study findings: The results of the 26-week toxicity study in dogs given acamprosate at doses of 250, 500, and 1000 mg/kg/day by oral gavage showed diarrhea in all treated dogs after each administration, with a dose-related increase in incidence and severity. The effect was reversible before each subsequent treatment. Several cardiac rhythm and conduction abnormalities at the highest dose were possibly related to acamprosate administration. These included one 2nd degree auriculo-ventricular heart block after the first dose, one 1st degree block before administration in week 13 in one male dog, one ventricular premature beat at lead II before administration in week 13 in another male dog, and several 2nd degree auriculo-ventricular blocks before administration in week 13 in one female dog. No findings were noted at week 26 and no changes in QT interval were observed. There was a dose-related increase in urinary calcium in all acamprosate-treated animals. The NOAEL was not established due to diarrhea and increased urinary calcium at the low dose. No definitive target organs of toxicity were identified. The doses studied (250-1000 mg/kg/day) represented 3.4X to 13.5X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis.

Toxicology Summary: A summary of the single dose studies conducted to evaluate acute acamprosate toxicity is presented in the following table.

Summary of Single Dose Toxicology Studies on Acamprosate

Species/Strain	Test Article Dose and Route (g/kg)	LD_{50}* (g/kg)	Clinical Signs	Reference
Mouse (CD1.VAF (IOPS)) n=10/sex/dose	Acamprosate 0,0.25,0.5,0.75,0.875,1.0 (IV)	M:0.707 F:0.819 C:0.771	≥0.75 g/kg:Convulsions, cardiac arrest 0.85 g/kg: decr body weights Deaths at ≥0.5g/kg due to cardiac arrest, assoc w/hard, contracted heart; auricle blood, GI liquid NOAEL:0.25g/kg	Study 90.03AOT. 001.SP2 Vol. 11
Mouse (CD1.VAF (IOPS)) n=10/sex/dose	Acamprosate 0,6,7,8,9,10 (PO)	M:8.06 F:8.64 C:8.37	≥7 g/kg: apathy ≥8 mg/kg: cardiac arrest, decr body wt and food consumption, deaths due to	Study 90.02AOT. 001.SP1

			cardiac arrest assoc with hard heart, pulmonary hyperemia, cranial blood NOAEL: 6 g/kg	Vol. 11
Mouse (Swiss OFI) n=5/sex/dose	Acamprosate 1-3(IP),6-10(PO),0.25-1(IV) Calcium chloride 0.7-1(IP),2-5(PO),.3-.5(IV) Homotaurine 6-7(IP),8(PO) Na acetylhomotaurinate 10(IP),8(PO)	C:0.72(IV) 1.5(IP) 7.7(PO) C:0.32(IV) 0.79(IP) 3.68(PO) C:6.2(IP) >8(PO) C:>10(IP) >8(PO)	Dose-related reduced motor activity, muscle spasms, hypotonia, bradycardia, ptosis, lower limb paralysis convulsions, GI tract congestion, deaths GI tract congestion. No effect No effect	Study 1100.22.02.84 Vol. 12
Rat (Sprague-Dawley) n=10/sex/dose	Acamprosate 0,.125,.25,.5,.75,1 (IV)	M:0.71 F:0.78 C:0.73	Convulsions, cardiac arrest before deaths	Study 90.01.AOT.001.RP2 Vol. 12
Rat (Sprague-Dawley) n=10/sex/dose	Acamprosate 0,5,5.75,6.6,7.6,8.75 (PO)	M:6.45 F:5.93 C:6.16	Transient apathy, diarrhea at all doses, hyperpnea, cardiac arrest	Study 90.04.AOT.001.RP3 Vol. 12
Rat (Sprague-Dawley) n=10/sex/dose, (15/sex in 1.25 and 1.5 g/kg IP acamprosate groups)	Acamprosate 1-1.75(IP),8-10(PO) Calcium chloride 1-2(IP),3-5(PO) Homotaurine 8(IP),8(PO) Sodium acetylhomotaurinate 8(IP),8(PO)	C:1.25(IP) 9.34(PO) C:1.15(IP) 3.60(PO) C:>8(IP) >8(PO) C:>8(IP) >8(PO)	IP: Reduced motor activity, muscular hypotonia, gen. paralysis, PO: Red. motor activ., dyspnea, salivation, cardiac arrest. None No effect No effect	Study 1100.22.02.84 Vol. 12
Rabbit (Burgundy tawny) n=5 males, 4 females	Acamprosate 15% IV infusion 0.7 ml/min until death Calcium chloride 10% IV inf. 0.7 ml/min until death	MLD:2.23 MLD:1.167	Hypotension, cardiac arrest before death Hypotension, cardiac arrest before death	Study 1100.22.02.84 Vol. 12
Rabbit (Burgundy tawny) n=5 males	Acamprosate 0.6 (PO)	-	Wet stools	Study 1602.26.11.86 Vol. 12

*M: male, F: female, C:combined, MLD: minimum lethal dose

Single dose toxicity was studied in mice, rats, and rabbits. Intravenous acamprosate administered to CD1 mice resulted in treatment-related convulsions, apathy, ataxia and injection site wounds, with deaths at doses of ≥500 mg/kg. The deaths were attributed to cardiac arrest, although no cause of death was definitely established. The deaths were associated with gross observations of hard, contracted heart, retention of blood in the auricles and aorta, and intestinal congestion and liquid contents. Acamprosate produced apathy, and decreased bodyweight gain and food

consumption at 7 and 8 g/kg, respectively, in mice by the oral route. Deaths by oral acamprosate at doses of 8 g/kg and higher were attributed to cardiac arrest. In another study in Swiss mice, acamprosate was administered by the intraperitoneal (IP, 1-3.0 g/kg), intravenous (IV, 0.25-1.0 g/kg) and oral (PO, gavage, 6-10 g/kg) routes. The results showed treatment-related reduction in motor activity, muscle spasms, generalized hypotonia and bradycardia by the IP route, reduced motor activity, ptosis, and paralysis of the lower limbs by the oral route, and agitation with convulsions and death by the IV route. The LD50 values in mice were 1.5 g/kg IP, 0.72-0.77 g/kg IV, and 7.70-8.4 g/kg PO.

In a single dose toxicity study in Sprague-Dawley rats given doses of 1.0-1.75 g/kg IP and 8.0-10.0 g/kg PO acamprosate, the clinical signs were reduced motor activity, muscular hypotonia, generalized paralysis, diarrhea, salivation, and deaths at the higher doses. The deaths occurred within 48-72 hours of dosing, and were correlated with GI tract congestion in the necropsy. The LD50 values in that study were 1.25 g/kg IP and 9.34 g/kg PO (38x the MRHD on a BSA basis). The gross necropsy showed blood-stained fluid in the digestive tract, and congestion of the intestinal mucosa. In another study, CD Sprague Dawley rats were administered acamprosate by IV injection at doses of 0.125-1.000 g/kg, and the animals were observed for 14 days. The NOAEL was 0.125 g/kg. In the surviving animals, injection site wounds were observed at 0.500-0.750 g/kg and convulsions followed by deaths at 1 g/kg. Body weights were reduced by >10% at 0.75 g/kg. The macroscopic examination showed splenomegaly in 1/10 males and 4/10 females, and discolored liver in 1/10 males and 2/10 females at 0.75 g/kg, without histopathologic changes in the microscopic examination. Spleen weights were increased at doses ≥0.5 g/kg in the males and 0.75 g/kg in the females, corresponding with hypertrophy of the white pulp and/or the red plug in the microscopic examination. The LD50 in that study was 0.71 g/kg in the males and 0.73 g/kg in the females. Oral (gavage) acamprosate, given to CD Sprague-Dawley rats at doses of 5-8.75 g/kg PO, resulted in transient apathy and diarrhea in all treated animals, and deaths at the higher doses. The oral LD50 was 6.45 g/kg in the male rats, 5.93 g/kg in the female rats, and 6.16 g/kg (25x the MRHD on a BSA basis) when evaluation of the male and female rats was combined.

In the Burgundy tawny rabbit, single intravenous infusion in a 15% solution (0.7 ml/min, 1.5-3.17 g/kg) resulted in decreased blood pressure, cardiac arrest and death in 3 of 9 rabbits tested. The minimum lethal dose was 2.23 g/kg IV. Oral acamprosate administration at single doses of 600 mg/kg (3-5 tablets at 333 mg/tablet) in rabbits resulted in wet stools and no deaths.

The preclinical multiple dose toxicity study results, with protocol summaries and treatment-related effects, are presented in the following table.

Summary of Repeated Dose Toxicology Studies on Acamprosate

Species/Strain	Acampro-sate Dose (mg/kg/d)	Dura-tion	Mortal-ity	Clinical Signs	Clinical Pathology	Organ Weight Pathol:Gross& Micro	NOAEL/ EL (mg/kg)	Refer-ence
Mouse (CD1 VAF) n=12/sex/dose	0,100,400 dietary	15 d	0	None	Not Determined	Not Determined	-	Study 91.05.A OT.001. SP3 Vol. 13
Mouse (CD1) 10/sex/dose	0,500,1000,1 500,2000	13 wk	0	Incr water consumption	Incr urinary Ca & P	1000: decr brain, heart, liver, spleen,	0/500	Study -

	dietary					testes wt , 2000: decr brain, heart wt		A.138/88 827 Vol. 13
Rat (Sprague-Dawley) 18/sex/dose	0,100,400 dietary	3 wk	0	Incr body wt gain in females, decr body wt gain in males	Plasma Ca determined only, No effect	Not Determined	-	Study 91.07.A OT.001. RP4 Vol. 13
Rat (Sprague-Dawley) 10/sex/dose	0, 500, 1000, 2000 dietary	13 wk	0	≥500: loose feces ≥1000 incr water consumption	≥500 decr urinary vol ≥1000 incr urinary CA	≥500: watery, pale GI contents ≥1000: decr liver wt 2000: incr adrenal wt , decr heart & ovary wts	0/1000	Study — A 139/8883 4 Vol. 14
Rat (Sprague-Dawley) 9/sex/dose	0,320,960, 2400 PO	90 d	0	Salivation, liquid diarrhea at high dose	None	≥960: adrenal wt , 2400: abs gonad wt , 2400 recovery: distended kidney tubule sections	0/320	Study 1097 Vol. 14
Rat (Sprague-Dawley) 15-30/sex/dose, reversibility: 5-10/sex/dose	0,320,960, 2400 PO	26 wk	5M & 17F at 2400	Dose related soft/liquid feces, transient ptyalism, water consumption, piloerection, hypothermia, incr water consumption,	320: urine acidity , incr urine protein &hemoglobin, 960: , urine acidity , urine protein & hemoglobin , 960,2400: incr blood urea N , Ca , serum P , incr urine Ca	960: incr heart, adrenal&kidney wts , cerebellum vacuolation 2400: heart& spleen, adrenal wt, degen renal tubulopathy, renal-cardiac-digestive-vascular calcifications, hyperkeratosis, stomach dysplasia, cardiac myolysis in rats that died	0/320	Study 602201 Vol. 15
Dog (Beagle) 3/sex/dose	0,25,100, 200 IV	4 wk	0	25-200: vomiting, salivation, swelling/indura-tion at injection site	200: incr serum Ca , decr P	No effect	0/25	Study 35191 Vol. 18
Dog (Beagle) 2/sex	1000 PO	4 wk	0	Liquid diarrhea, decr body wts (5-7%)	None	No effect	-	Study 509215 Vol. 17
Dog (Beagle) 4/sex	0,250,500, 1000 PO	26 weeks	0	Dose related diarrhea, severe at HD Cardiac rhythm/conduction abnormalities in several HD dogs	Dose related incr urine Ca , decr serum P, incr serum Cl at HD	No effect	0/250	Study 509215 Vol. 17
Monkey (Macaque) 2 male, 1 female	1000 PO	7 days	0	Diarrhea, decr body wts (5-6%)	None	No effect	-	Study 1605 Vol. 18

In CD-1 VAF mice administered target doses of 100 and 400 mg/kg/day (0.2x and 0.8x the MRHD on a BSA basis) by admixture in the diet for 2 weeks, mean acamprosate intake was 125-137 and 534-564 mg/kg/day in the low and high dose animals, respectively. Wounds and hair loss were observed in the high dose groups, and increased food consumption and body weight

gains in both dose groups. Plasma sampling demonstrated that acamprosate was well absorbed in mice; steady plasma levels indicated that sampling can be conducted at any time of day. In a 13-week oral (dietary) toxicity study in CD-1 mice (doses of 500, 1000, 1500 and 2000 mg/kg/day; 1x, 2x, 3x, and 4x the MRHD on a BSA basis), findings included increased water consumption at doses from 1000-2000 mg/kg/day, and increased urinary calcium and phosphorus at 2000 mg/kg/day. Brain, heart, liver, spleen and testes weights were slightly decreased in males at doses of 1000-2000 mg/kg/day. In the female mice, brain weights were decreased at 1000 and 2000 mg/kg/day and heart weights were decreased at 2000 mg/kg/day. The MTD was determined to be > 2000 mg/kg.

Subchronic toxicity of acamprosate was studied in Sprague Dawley rats treated by dietary administration at doses of 100 and 400 mg/kg/day (0.4x and 1.6x the MRHD on a BSA basis) for 3 and 13 weeks, and by oral gavage for 3 months. In the 3-week study, there was a dose-related increase in body weight gain in the females compared to controls, and a decrease in body weight gain in the males. Plasma acamprosate was not detected in the rats at 100 mg/kg/day; mean plasma concentrations in the males were 0.98-3.33 mg/l and in the females were 0.75-4.29 mg/l at 400 mg/kg/day; peak plasma levels occurred in the morning. A NOAEL was not established in this study. Dietary administration of acamprosate for 13 weeks at doses of 500, 1000, and 2000 mg/kg/day (2x, 4x, and 8x the MRHD on a BSA basis) resulted in loose feces primarily at the two upper doses, increased water consumption, decreased urinary volume in females, and increased urinary Ca. The necropsy showed decreased liver weights in the males, increased adrenal weights in males, and decreased ovarian and heart weights in females. Treatment-related firm contents in the ileum, watery distension and pale contents in the cecum, and soft, pale colon contents were observed in males at 500 mg/kg/day, and males and females at 1000 and 2000 mg/kg/day. The MTD in this study was 1000 mg/kg/day based upon renal effects. A NOAEL was not identified in this study.

Acamprosate administration by daily oral intubation, at doses of 320, 960, and 2400 mg/kg/day (1.3x, 4x, and 10x the MRHD on a BSA basis) for 90 days in rats, resulted in salivation and liquid diarrhea in 1 high-dose animal. Findings included reversible increases in gonad weight at 2400 mg/kg/day, and adrenal weight at 960 and 2400 mg/kg/day in the male rats. The histopathology examination showed distended kidney tubule sections from coagulum accumulations, attributed to early senile nephrosis in 3 high dose recovery rats. A NOAEL could not be identified because a full histopathology assessment was not conducted. In a chronic (26-wk) oral (gastric intubation) toxicity study (320, 960, and 2400 mg/kg/day; 1.3x, 4x, and 10x the MRHD on a BSA basis). Drug related deaths at 2400 mg/kg/d in male (17%) and female (57%) rats occurred between the weeks of 15 and 26 of dosing. Treatment-related findings included clinical signs (piloerection, subdued behavior, hypothermia, sudden weight loss, ptyalism and soft feces) and a dose-related increase in water consumption. Clinical chemistry changes included increases in alkaline phosphatase in the females and blood urea nitrogen, increased serum and urinary calcium, and acidified urine. The histopathology identified the liver, kidney, heart, lung, thymus, spleen, stomach, duodenum, cecum as target organs in the animals that died. Similar organs were identified in surviving animals in addition to the bladder, brain, and adrenal. Associated gross findings included decreases in heart and spleen weight, and increases in adrenal and kidney weight, and gastrointestinal system effects. The NOAEL was not identified in this study due to an increased incidence of dyskeratosis and inflammation of the stomach and vacuolation of the cerebellum at the mid-dose and the lack of histopathology evaluation at the

low dose.

Subchronic (4-week) toxicity in Beagle dogs was evaluated by the intravenous and oral routes. Intravenous treatment (20, 100 and 200 mg/kg/day) resulted in vomiting and injection site swelling and induration, with a dose-related increase in incidence. Treatment-related findings included clinical signs (salivation, tremors, chewing, and agitation), changes in clinical chemistry (increase in calcium and decrease in phosphorus levels), and macroscopic and microscopic changes (induration, swelling, hemorrhagic infiltration, periphlebitis and granulomatous inflammation at the injection site). A NOAEL was not identified due to vomiting and injection site effects in a small number of low-dose dogs. The plasma acamprosate measurements showed lower levels in females compared to males from 1 hour to 4 hours after dosing, suggesting higher clearance of acamprosate in the females. In a preliminary oral toxicity study, beagle dogs were given acamprosate at 1000 mg/kg/day (13.5x the MRHD on a BSA basis) by gastric intubation to determine the MTD for the 26-week study. A NOAEL was not identified due to liquid diarrhea in all animals after dosing and slight, reversible body weight reduction in the male dogs; no histologic assessment was performed.

A chronic (26-week) oral (gavage) toxicity study in dogs given acamprosate at doses of 250, 500, and 1000 mg/kg/day (3.4X, 6.8X, and 13.5x the MRHD on a BSA basis) again showed diarrhea in all drug-treated dogs, with a dose-related increase in incidence and severity; the effect was reversible before each subsequent treatment. Cardiac rhythm and conduction abnormalities (1st and 2nd degree auriculo-ventricular heart block, ventricular premature beat at lead II) that were possibly related to acamprosate administration were observed at the high dose after the first dose and at week 13. No findings in QT interval were noted and no findings were observed at week 26. There was a dose-related increase in urinary calcium in all acamprosate-treated animals. The NOAEL was not established due to diarrhea and increased urinary calcium at the low dose although no definitive target organs of toxicity were identified. The doses studied (250-1000 mg/kg/day) represented 3.4X to 13.5X the MRHD of 1998 mg/day in a 50 kg patient on a BSA basis.

In cynomolgus monkeys, 7-day oral acamprosate administration by gavage at 1 g/kg/day (8x the MRHD on a BSA basis) resulted in liquid diarrhea in all animals throughout the dosing period. A slight decrease in body weights was probably a result of the liquid diarrhea. The NOAEL cannot be determined because clinical pathology and histopathologic examination were not performed.

Toxicology conclusions: The toxicity following single dose administration is considered low in mice and rats following intravenous, intraperitoneal and oral administration. The oral median lethal doses are approximately 10x higher than the IV LD_{50} values suggesting poor oral bioavailability in rodents. Oral and IV LD_{50} values in mice were 7700-8370 and 720-771 mg/kg respectively, and in rats were 6160-9340 and 730 mg/kg respectively.

Chronic dosing in rats produced death at 2400 mg/kg and identified the liver, kidney, heart, lung, thymus, spleen, stomach, duodenum, cecum, bladder, brain, and adrenal as target organs of toxicity. Chronic dosing in dogs up to 1000 mg/kg did not identify target organs of toxicity although potential drug-related changes in cardiac rhythm and conduction abnormalities were

noted. In dogs and monkeys, acamprosate caused diarrhea.

Histopathology Inventory for NDA # 21-431

Study	138/88827	139/88834	1097	602201. 1986	35191	509215
Species	Mouse 13wk Oral	Rat 13wk Oral	Rat 3mo Oral	Rat 26wk Oral	Dog 28-d IV	Dog 26wk Oral
Adrenals	X*	*	X*	X*	X*	X*
Aorta	X			X		X
Bone Marrow smear				X	X	X
Bone (femur)	X			X	X	X
Brain	X*	*		X*	X*	X*
Bronchi (mainstem)					X	
Cecum	X		X	X	X	X
Cervix						
Colon	X		X	X	X	X
Duodenum	X		X	X	X	X
Epididymis				X	X	X
Esophagus	X			X	X	X
Eye	X			X	X	X
Fallopian tube						
Gall bladder	X				X	X
Gross lesions				X	X	X
Harderian gland	X					
Heart	X*	X*	X*	X*	X*	X*
Ileum	X		X	X	X	X
Injection site					X	
Jejunum	X			X	X	X
Kidneys	X*	X*	X*	X*	X*	X*
Lachrymal gland	X					
Larynx	X					
Liver	X*	*	X*	X*	X*	X*
Lungs	X			X	X	X
Lymph nodes, cervical	X					
Lymph nodes mandibular				X		
Lymph nodes, mesenteric	X			X	X	X
Lymphnode, submaxillary					X	X
Mammary Gland	X			X	X	X
Nasal cavity	X					
Optic nerves					X	
Ovaries	X*	*		X	X*	X*
Pancreas	X			X	X	X
Parathyroid	X			*	X	X*
Peripheral nerve						
Pharynx	X					
Pituitary	X*	*		X*	X*	X*
Prostate	X			X	X	X
Rectum	X			X		X
Salivary gland	X			X	X	X
Sciatic nerve	X			X		X
Seminal vesicles	X			X		
Skeletal muscle	X			X	X	X
Skin	X			X	X	X
Spinal cord	X			X	X	X

Spleen	X*	*	X*	X*	X*	X*
Sternum	X					
Stomach	X			X	X	X
Testes	X*	*	X*	X*	X*	X*
Thymus	X			X	X	X
Thyroid	X*	*	X	X*	*X	X*
Tongue	X					
Trachea	X			X	X	X
Urinary bladder	X			X	X	X
Uterus	X*	*		X	X	X
Vagina	X					
Zymbal gland	X					

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

Study title: MUTAGENICITY STUDY IN SALMONELLA TYPHIMURIUM HIS ACCORDING TO THE B.N. AMES TECHNIQUE ON CALCIUM ACETYLHOMOTAURINATE (ACAMPROSATE)

Key findings:

- Acamprosate negative for mutagenicity in *Salmonella typhimurium* HIS (Ames test) at concentrations of 50-5000 µg/plate under the conditions tested. Tester strains with the AT base pair at the primary reversion site were not evaluated.

Study no: 83058, 1983
Study type: Mutagenicity: to detect induction of DNA base pair substitution and frameshift mutations (Ames *et al.*, Mutation Res.31:347-364, 1975)
Volume # 19, and page #: 1
Conducting laboratory and location: [redacted]

Date of study initiation: November 29, 1983
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate (AOTA Ca), **lot #** 1395/8, **radiolabel** none, **and % purity:** not provided

Formulation/vehicle: Test article in distilled water

Methods:

Strains/species/cell line: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100. The selected strains did not include *E. coli* WP2 or *S. typhimurium* strain TA 102 as recommended in OECD guidelines.

Dose selection criteria:

Basis of dose selection: Up to the highest dose normally studied in laboratory (5 mg/plate)

Range finding studies: Test article added to bacterial strains in histidine-biotin-rich medium at 5, 1.5, 0.5, 0.15 and 0.05 mg/plate with incubation period 24 hours; bacteriostatic activity < 75% at highest concentration, and 4 lower doses selected in geometric ratio

Test agent stability: No precipitation observed

Metabolic activation system: 0.5 ml hepatic microsomal enzymes (S9) prepared from rats pre-treated with AROCLOR 1254 according to method of Ames *et al.* (Proc. Nat. Acad. Sci. USA 70:2281-5, 1973).

Controls:

Vehicle: Sterile distilled water

Negative controls: Sterile distilled water

Positive controls:

Salmonella typhimurium strain	Reference Product Used as Positive Control Without Metabolic Activation		Reference Product Used as Positive Control With Metabolic Activation	
	Chemical	Dose (mcg/plate)	Chemical	Dose (mcg/plate)
TA 1535	*B*-Propiolactone	50	2-Anthramine	5
TA 1537	Hycanthone Methane-sulphonate	50	2-Anthramine	5
TA 1538	2-Nitrofluorene	2	2-Anthramine	5
TA 98	2-Nitrofluorene	2	2-Anthramine	5
TA 100	Sodium Azide	20	2-Anthramine	5

Comments: The selected controls were appropriate; Positive controls tested in parallel with and without S9; Assay was conducted in triplicate, replicated 2x

Exposure conditions:

Incubation and sampling times: Incubation period 48 hours

Doses used in definitive study: 50, 150, 500, 1500, 5000 mcg/plate

Study design: AOTA Ca dissolved in water, 0.1 ml added to moist agar (2 ml/plate) with histidine and biotin, superfusion at 45°C, 0.1 ml culture added, incubated 18 h at 37°C with and without S9, homogenized and poured into Petri dishes on solidified agar layer at 3 plates per concentration of AOTA Ca per strain, and incubated 48 h at 37°C; number of colonies of prototrophic mutants determined with colony counter, R ratio (mean number mutants per plate with AOTA Ca / mean number mutants per plate without AOTA Ca) determined

Analysis:

No. of replicates: 2

Counting method: Colony counter

Criteria for positive results: R ratio > 2 for one or several doses with a dose-effect in one section of the range of doses studied in 2 independent tests

Summary of individual study findings:

Study validity:

1. Test product sterility (no colony visible at highest dose after incubation 48 hours at 37°C)
2. Frequency of spontaneous back mutations (control without solvent) within limits of standard control values
3. Frequency of spontaneous back mutations with solvent not significantly different from frequency of back mutations in absolute control for each test
4. Frequency of back mutations by reference products in each strain greater than lower standard limit with and without S9: Positive response by the positive controls
5. The use of strains *E. coli* WP2 and *S. typhimurium* TA 102 was not included
6. Dose selection was adequate: bacteriostatic activity less than 75% at the highest concentration

Study outcome:

The results of the dose finding study showed low to no bacteriostatic activity by the test article at up to 5000 mcg/plate. Therefore 5000 mcg/plate was chosen to be the highest dose for the

mutagenicity study. No increase in R ratio (mean # mutants with AOTA-Ca / mean # mutants by solvent alone) to levels greater than 2 at any dose up to 5000 mcg/plate in any strain tested, with and without metabolic activation with S9 mix. Increase in the R ratio by the positive controls ranged from 20.5-258.1. In agreement with the sponsor's conclusion, AOTA-Ca was negative in the Ames test under the conditions of this study.

Study title: STUDY TO DETERMINE THE ABILITY OF ACAMPROSATE TO INDUCE MUTATION IN FOUR HISTIDINE-REQUIRING STRAINS OF SALMONELLA TYPHIMURIUM AND TWO TRYPTOPHAN-REQUIRING STRAINS OF ESCHERICHIA COLI

Key findings:

- Acamprosate was negative in the Ames test in 4 strains of histidine-dependent *Salmonella typhimurium* and 2 strains of tryptophan-dependent *Escherichia coli* at concentrations up to 5000 mcg/plate under the conditions tested

Study no: 537/50
Study type: Mutagenicity: to detect induction of DNA base pair substitution and frameshift mutations (Ames *et al.*, Mutation Res.31:347-364, 1975)
Volume # 19, and page #: 31
Conducting laboratory and location: [redacted]
Date of study initiation: **March 26, 1993**
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate (AOTA Ca), **lot #** OTA 3049, **radiolabel** none, **and % purity:** [redacted]

Formulation/vehicle: Test article dissolved in sterile purified water ([redacted])

Methods:

Strains Studied in the Ames Test

Strain		Type of Mutation	Mutant Gene
S. typhimurium	TA98	Frame-shift	histidine
S. typhimurium	TA100	Base-pair substitution	histidine
S. typhimurium	TA1535	Base-pair substitution	histidine
S. typhimurium	TA1537	Frame-shift	histidine
E. coli	WP2 pKM101	Base-pair substitution	tryptophan
E. coli	WP2 uvrA pKM101	Base-pair substitution	tryptophan

Dose selection criteria:

Basis of dose selection: Maximum required concentration in the absence of cytotoxicity in the range-finding study except for slight toxicity at 5000 mcg/plate
Range finding studies: 3 toxicity range-finding studies were conducted using final concentrations of 8, 40, 200, 312.5, 625, 1000, 1250, 2000, 2500, 3000, 4000 and 5000 mcg/plate with and without metabolic activation with S9 mix, incubated for 3 days at 37°C. The results showed no evidence of toxicity at any concentration up to

4000 mcg/plate, and slight toxicity (slight thinning of background bacterial lawn in all strains except TA100) at 5000 mcg/plate

Test agent stability: No precipitation observed

Metabolic activation system: 0.5 ml 10% Aroclor-induced rat liver post-mitochondrial fraction (S9)

Controls:

Vehicle: Sterile purified water [redacted]

Negative controls: Sterile purified water [redacted]

Positive controls:

Positive Controls Used in the Ames Test

Chemical	Source	Stock concentration (mcg/ml)	Final concentration (mcg/plate)	Strain	S9
2-nitrofluorene (2NF)	[redacted]	500	50.0	TA98	-
Sodium azide (NaN_3)		20	2.0	TA100 TA1535	- -
9-aminoacridine (AAC)		500	50.0	TA1537	-
4-nitroquinoline 1-oxide (NQO)		40 20	4.0 2.0	WP2 pKM101 WP2uvrA pKM101	- -
2-aminoanthracene (AAN)	[redacted]	50	5.0	TA98 TA100 WP2 uvrA pKM101	+

Comments: Controls were appropriate

Exposure conditions:

Incubation and sampling times: Incubation time 3 days at 37°C

Doses used in definitive study: The following acamprosate concentrations were used in the mutagenicity study:

Acamprosate Concentrations Used in the Ames Test

Experiment	S9	Concentration of treatment solution (mg/ml)	Final Concentration (mcg/plate)
1	- and +	0.08 0.40 2.00 10.00 50.00	8 40 200 1000 5000
2	- and +	3.125 6.250 12.50 25.00 50.00	312.5 625 1250 2500 5000
3	+	10.00 20.00 30.00 40.00 50.00	1000 2000 3000 4000 5000

Analysis:

No. of replicates: Study was conducted in triplicate

Counting method:[ ] Colony Counter or manual (Experiment 3)

Criteria for positive results:

1. Assay was valid
2. Statistically significant increase in revertants ($p \leq 0.01$) and significant dose correlation
3. Positive responses reproducible

Summary of individual study findings:

Study validity:

1. Mean negative control count within historical range
2. Positive control chemicals induced significant increases in revertants allowing discrimination between strains and active S9 preparation
3. 5% or fewer plates lost through contamination
4. Appropriate strains were used
5. Dose selection was adequate

Study outcome:

The solvent controls were within normal historical range in numbers of revertants. There was a small increase in revertant numbers in the Escherichia coli strain WP2 pKM101 in the presence of metabolic activation in experiment 1 (127.7 vs. 94.2 in the control plates, $p<0.05$) at 5000 mcg/plate, and in experiment 2 without metabolic activation (69.3 vs. 49 in the controls, $p<0.05$) at 2500 but not at 5000 mcg/plate, and with metabolic activation (94.0 at 2500 and 106.0 at 5000 mcg/plate compared to 68.0 in the controls, $p<0.01$ and $p<0.001$ respectively). No statistically significant increase in revertants were observed in this strain in experiment 3 at concentrations from 1000-5000 mcg/plate, nor in any other strain in the 3 experiments at up to 5000 mcg/plate. The numbers of revertant colonies for the strain WP2 pKM101 were within historical range (22-86 without S9 and 43-115 with S9) in experiment 2 but slightly above the upper limit in experiment 1. The increase in revertants at the high dose in strain WP2 pKM101 are not considered to be the result of mutagenic activity because acamprosate was cytotoxic at the high dose, the increase was within the range found in the historical data in all but one case, the increase lacked a dose-response, and was not reproducible in the third experiment. Also, the increases did not achieve the commonly used criteria for a positive test of 2- to 3-fold increase in mean revertants. Thus, acamprosate was negative under the conditions tested in this assay, in concurrence with the sponsor's conclusion.

Study title: MUTAGENICITY STUDY USING THE HPRT LOCUS MUTATION TECHNIQUE IN CHINESE HAMSTER V79 CELLS (RESISTANCE TO 6-THIOGUANINE) ON THE PRODUCT CALCIUM ACETYLHOMOTAURINATE (ACAMPROSATE)

Key findings:

- Increased number of mutants at 300 mcg/ml in Test 1 and at 100, 1000, 3000 (but not 300) mcg/ml in Test 2 without metabolic activation; No increase in number of mutants in Test 3 at

any concentration without metabolic activation; No increase in number of mutants in any test, at any concentration with metabolic activation

- The highest concentration tested (3000 mg/ml) was not adequate in the absence of precipitation or cytotoxicity
- The mutagenic potential for acamprosate cannot be ruled out; the assay should be repeated using appropriate doses

Study no: 85065
Study type: Mutagenicity: to detect mutation (resistance to 6-thioguanine) at the HPRT locus in mammalian cells
Volume # 19, and page #: 90
Conducting laboratory and location: [redacted]

Date of study initiation: September 24, 1985
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Calcium N-Acetylhomotaurinate (AOTA Ca), **lot #** 1395/13, **radiolabel** none, **and % purity:** [redacted]

Formulation/vehicle: Dulbecco H16 medium

Methods:

Strains/species/cell line: Chinese Hamster V79 cells
Dose selection criteria:
Basis of dose selection: Solubility limit 3000 mcg/ml, cytotoxicity study at up to 3000 mcg/ml with and without S9 incubated for 3 hours
Range finding studies: No cytotoxicity to V79 cells at doses of up to 3000 mcg/ml, with and without metabolic activation; Sponsor stated that product is insoluble in H16 medium at concentrations in excess of 3000 mcg/ml, no precipitate was reported in the range finding studies
Test agent stability: Solubility limit 3000 mg/ml in Dulbecco H16 medium vehicle
Metabolic activation system: 25% culture medium replaced with 25% S9 mix (0.1 ml S9/ml mix) consisting of hepatic microsomes obtained from livers of rats treated with Aroclor 1254 for 3 h

Controls:
Vehicle: Dulbecco H16 medium
Negative controls: Dulbecco H16 medium
Positive controls: Ethylmethane sulphonate without S9; dimethylnitrosamine with S9
Comments: Appropriate controls used; dosing may not have been adequate, as precipitate was not reported at 3000 mcg/ml
Exposure conditions:
Incubation and sampling times: Cells were exposed to the negative and positive control, and test articles for 3 hours
Doses used in definitive study: 0, 100, 300, 1000, 3000 mcg/ml

Study design: The exposed cells were subcultured (200 cells/plate) to determine reproduction rate (6 plates/dose) and to allow phenotype expression of mutation (3 plates/dose). The cells were then exposed to 6-thioguanine to reveal resistant (mutated at HPRT locus) cells 3 hours after subculture, and incubated at 37°C, fixed in methanol and stained 8-10 days later for examination. Reproduction rate was defined as mean number of treated cells surviving / mean number of control cells surviving x 100. Mutation level per 10^6 cells was defined as mean number of mutant cells x 2 / mean number of cells surviving per 200.

Analysis:

No. of replicates: 2x

Counting method: Mutants were counted under a binocular magnifier.

Criteria for positive results: Induction of 3x increase in mutation frequency compared to spontaneous mutants

Summary of individual study findings:

Study validity:

1. Results in the negative solvent controls comparable to those in the historical controls for the laboratory
2. Results in the positive control samples demonstrated sensitivity of cell line to the HPRT locus mutation and efficacy of metabolic activation system
3. Dosing may not have been adequate as OECD guidelines recommended that the high dose should produce some precipitation when dosing is limited by solubility

Study outcome: The results are presented in the following table:

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Results of the Mutagenicity Study in Chinese Hamster V79 Cells

Test*	Number of Mutants per 10^6 Cells (mean of 2 assays)					
	Neg Control	Pos Control*	AOTA-Ca 100 mcg/ml	AOTA-Ca 300 mcg/ml	AOTA-Ca 1000 mcg/ml	AOTA-Ca 3000 mcg/ml
Absence of Metabolic Activation (-S9)						
Test 1	7	**1335**	11.7	**41.6 (5.9x)**	9.9	11.3
Test 2	7.3	**797**	**28.7 (3.9x)**	3.1	**36 (4.9x)**	**38.9 (5.3x)**
Mean Tests 1 and 2	7.2	**1066**	20.2	22.4	23	25.1
Ratio (Tests 1 and 2)	-	**148**	2.8	3.1	3.2	3.5
Test 3	14.5	**1432**	3	5	4.6	6.5
Ratio Test 3	-	**98.8**	0.2	0.3	0.3	0.4
Mean Tests 1, 2, and 3	9.6	**1188**	14.1	16.6	16.8	18.9
Ratio (Tests 1, 2, and 3)	-	**124**	1.5	1.7	1.7	2
Presence of Metabolic Activation (+S9)						
Test 1	19.1	**326**	8.5	16.7	5.9	24.4
Test 2	14.9	**319**	19.3	17.5	22.1	0
Mean Tests 1 and 2	17	**324**	13.9	17.1	14	12.2
Ratio (Tests 1 and 2)	-	**19.1**	0.8	1	0.8	0.7
Test 3	9.3	**204**	10.2	2.9	6.3	1.3
Ratio Test 3	-	**21.9**	1.1	0.3	0.7	0.1
Mean Tests 1, 2, and 3	14.4	**284**	12.7	12.4	11.4	8.6
Ratio (Tests 1, 2, and 3)	-	**19.7**	0.9	0.9	0.8	0.6

*Ratio = mean number of mutants in the treated group / mean number of mutants in the control group; Positive controls were EMS (1 mg/ml) in absence of S9; DMN (1 mg/ml) in presence of S9

No statistically significant increase in the number of mutants was observed in the cells treated with the solvent control, Dulbecco H16 medium, alone. The number of mutants was increased (5.9x vs spontaneous mutants) by acamprosate at 300 mcg/ml in the absence of metabolic activation with S9 mix in Experiment 1. In Experiment 2, the number of mutants were significantly increased at 100, 1000 and 3000 mcg/ml (3.9x, 4.9x, and 5.3x vs. spontaneous mutation rate, respectively), but not at 300 mcg/ml, in the absence of metabolic activation. No increases were observed in mutations by acamprosate in the third experiment, conducted in the absence of metabolic activation, or in all three experiments in the presence of metabolic activation.

Although the highest dose used was claimed to be limit of solubility, no precipitate was reported for this or higher doses. Therefore, dosing appears to be inadequate according to current standards. Also, because of the positive findings, the mutagenic potential of acamprosate cannot be ruled out. The sponsor concluded that acamprosate did not present any mutagenic activity detectable by the point mutation test at the HPRT locus in Chinese Hamster V79 cells. The

finding should be further evaluated by the sponsor.

Study title: GENOTOXICITY STUDY INVESTIGATING CHROMOSOME ABERRATIONS BY METAPHASE ANALYSIS IN HUMAN LYMPHOCYTES ON THE PRODUCT CALCIUM ACETYLHOMOTAURINATE (ACAMPROSATE)

Key findings:

- No clastogenicity observed by *in vitro* metaphase analysis in human lymphocytes
- Dosing was inadequate without metabolic activation as the high dose was limited by the sponsor due to solubility but did not cause precipitation in the current assay
- The incubation time with metabolic activation was inadequate (1 hour) according to current OECD guidelines
- The assay should be repeated using appropriate doses and incubation times.

Study no: 86002
Study type: Clastogenicity (chromosome aberrations) *in vitro* in human lymphocytes
Volume # 19, and page #: 124
Conducting laboratory and location: [redacted]

Date of study initiation: October 16, 1985
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Calcium N-Acetylhomotaurinate (AOTA Ca), **lot #** 1395/13, **radiolabel** none, **and % purity:** [redacted]

Formulation/vehicle: AOTA Ca dissolved in RPMI culture medium

Methods:

Strains/species/cell line: Human lymphocytes from healthy subjects (1 male and 1 female)

Dose selection criteria:

Basis of dose selection: Sponsor cited that solubility prohibited the use of concentrations > 300 mcg/ml in RPMI culture medium, no data on solubility submitted; cytotoxicity (see under Range finding studies below)

Range finding studies: Mitotic index determined at the following concentrations: 3, 10, 30, 100, 300 mcg/ml for 24 hours with and for 1 hour without S9; Acamprosate was highly cytotoxic at 300 mcg/ml in the presence of metabolic activation, based on mitotic index of 14.6% control value (>50% reduction of mitotic index). The results of the mitotic index determination are presented in the following table:

Results of Cytotoxicity Testing in the Chromosome Aberration Assay

Test Article	Concentration (mcg/ml)	Slide Number	Mitotic Index (per slide)	Mitotic Index (mean)	Percent Control
RPMI (- S9)	0	684	16	14	Reference
	0	694	12		
RPMI (+ S9)	0	704	36	27.5	Reference
	0	713	18		
Mitomycin C (-S9)	0.25	686	5	6	42.9%
	0.25	696	7		
Cyclophosphamide (+S9)	75	705	6	4	14.6%
	75	714	2		
AOTA-Ca (-S9)	300	687	16	12.5	89.3%
	300	697	9		
AOTA-Ca (+S9)	300	706	3	4	14.6%
	300	715	5		
AOTA-Ca (-S9)	100	688	18	14.5	103.6%
	100	698	11		
AOTA-Ca (+S9)	100	707	28	18	65.5%
	100	718	8		
AOTA-Ca (-S9)	30	689	15	14.5	103.6%
	30	699	14		
AOTA-Ca (+S9)	30	708	32	23	83.6%
	30	717	14		
AOTA-Ca (-S9)	10	690	18	15	107.1%
	10	700	12		
AOTA-Ca (+S9)	10	709	28	25	90.9%
	10	719	22		
AOTA-Ca (-S9)	3	691	14	16	114.3%
	3	701	18		
AOTA-Ca (+S9)	3	710	40	30	109.1%
	3	719	20		

Test agent stability: Not provided

Metabolic activation system: Aroclor 1254 induced rat microsomal enzymes (10%, 0.1 ml S9 per ml mix)

Controls:

Vehicle: RPMI culture medium

Negative controls: RPMI culture medium

Positive controls: Mitomycin C (0.25 mcg/ml) without S9; Cyclophosphamide (75 mcg/ml) with S9

Comments: Controls acceptable

Exposure conditions:

Incubation and sampling times: Cells incubated 48 hours, treated (duration 24 hours without S9, 1 hour and then replacement of medium with S9), then colcemide added 2 h before harvesting to arrest cells in metaphase (22 hours after treatment) and harvested 2 hours later. Incubation time with acamprosate in the presence of S9 (1 h) was inadequate according to current OECD guidelines

Doses used in definitive study: 30, 100, 300 (limit of solubility in RPMI culture medium) mcg/ml without S9; 10, 30, 100 (limit of cytotoxicity with S9) mcg/ml with S9; dosing inadequate in the absence of metabolic activation as no precipitation was observed at the high dose

Study design: Isolated lymphocytes induced to divide in culture by phytohemagglutinin, then exposed to control medium, acamprosate, or positive control articles; cell division blocked during metaphase by colcemide, cells fixed, smeared onto slides and stained; chromosomes from 200 cells per dose examined microscopically (x1000) for chromosome gaps, breaks, numerical aberrations (aneuploidy and polyploidy), and number of cells altered with and without gaps according to OCDE Guideline for testing of Chemical Genetic Toxicology (26 May 1983, No. 473, pp. 1-6).

Analysis:

No. of replicates: 2x

Counting method: See under Study design above

Criteria for positive results: Statistically significant increase in number of chromosome aberrations compared to negative controls

Summary of individual study findings:

Study validity:

1. Incidence of gaps and breaks in the negative and positive control groups within historical control ranges for the laboratory
2. Statistically significant increase in gaps or breaks in the positive control cells compared to negative controls
3. Adequate controls used
4. Incubation time with acamprosate in presence of S9 (1h) was inadequate
5. Dosing without metabolic activation was inadequate in the absence of precipitation and or cytotoxicity

Study outcome: There were no significant increases in chromosome or chromatid gaps or breaks by acamprosate at any dose up to 300 mcg/ml without metabolic activation. The number of gaps per cell was increased by acamprosate at 100 mcg/ml (7 and 11 in two slides, compared to 1 and 5 in the two control slides) in the presence of metabolic activation. In the absence of breaks and other indices of clastogenicity such as increased number of cells with aberrations and increased number of aberrations, the increase in gaps is probably due to cytotoxicity by acamprosate. The positive control articles mitomycin C and cyclophosphamide significantly increased the incidence of chromatid gaps, acentric chromosomes, chromatid fragments and exchanges, and total number of breaks. Thus, acamprosate tested negatively for clastogenicity at doses up to 300 mcg/ml without S9 and 100 mcg/ml with S9 under the conditions tested. This conclusion is in agreement with the sponsor's. However, the dosing without metabolic activation and incubation time used in this study do not appear to be valid.

Study title: MUTAGENICITY STUDY IN THE MOUSE USING THE MICRONUCLEUS TEST ON THE PRODUCT CALCIUM ACETYLHOMOTAURINATE (ACAMPROSATE)

Key findings:

- Acamprosate was negative for clastogenicity in the micronucleus test in mice at doses up to 6000 mg/kg/d given on 2 consecutive days (time 1 and time 0 + 24 h).

Study no: 84008
Study type: Clastogenicity, *in vivo*: induction of chromosome fragmentation in polychromatic bone marrow erythrocytes
Volume # 19, and page #: 155
Conducting laboratory and location: [redacted]
Date of study initiation: December 5, 1983
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Calcium N-acetylhomotaurinate (AOTA Ca), **lot #** 1395/8, **radiolabel** none, **and % purity:** Not provided

Formulation/vehicle: Distilled water

Methods:

Strains/species/cell line: OF1 [redacted] male and female mice (20-25 g)
Dose selection criteria:
Basis of dose selection: Preliminary toxicity test: MTD 6000 mg/kg x 2, due to 100% deaths at 12000 mg/kg x 2 after 72 hours
Range finding studies: Mice (3/sex/dose, weights 20 g) were administered acamprosate in distilled water by gavage (25 ml/kg) at oral doses of 3000, 6000, and 12000 mg/kg daily for 2 consecutive days. Mortality observations for 72 hours showed 0% mortality at 3000 and 6000 mg/kg/d, and 100% mortality at 12000 mg/kg/day. The MTD for the micronucleus test was considered 6000 mg/kg/day, by the sponsor. The MTD could be higher (<12000 mg/kg/day), but 6000 mg/kg/day exceeds the limit dose of 2000 mg/kg required for this assay.
Test agent stability: Stability and precipitation data not provided
Metabolic activation system: NA
Controls:
Vehicle: Distilled water
Negative controls: Distilled water
Positive controls: Cyclophosphamide 2 x 50 mg/kg IP (CPA)
Comments: Controls were appropriate
Exposure conditions:
Incubation and sampling times: Treatment times: 0 and 24 hours; bone marrow sampling times 48 and 72 hours (24 and 48 hours after the second dose)
Doses used in definitive study: 3000 and 6000 mg/kg/day x 2 days
Study design: 10 mice/sex/dose/timepoint (6/sex/timeoint positive controls) were

treated and sacrificed as described above, femurs removed, bone marrow extracted and smeared onto slides with appropriate stain; the marrow was examined for number of polychromatic erythrocytes with one or more micronuclei (at least 1000 per animal); 2000 polychromatic erythrocytes were observed per animal (2 observers each examined 1000 cells)

Analysis:

No. of replicates: 2

Counting method: Microscopic examination as described under Study Design above

Criteria for positive results:

Statistically significant increase in number of polychromatic erythrocytes with micronuclei compared to controls

Summary of individual study findings:

Study validity:

1. Incidence of micronucleated PCE in vehicle and positive control groups within historical control ranges for the laboratory
2. Significant increase in frequency of micronucleated PCE in positive control group
3. Report provided no information on proportion of immature erythrocytes among total erythrocytes

Study outcome:

No significant increase in number of polychromatic erythrocytes with micronuclei by negative control (mean 2.3 and 1.8 in males and females, respectively) and acamprosate (mean 1.9 and 2.3 at 3000 and 6000 mg/kg/day at 48 hours, respectively, and 1.35 and 1.15 at 3000 and 6000 mg/kg/day at 72 hours, respectively). Mean numbers of micronuclei in the positive control groups were 20.8 and 21.7 at 48 and 72 hours, respectively. Thus, the test drug was not clastogenic under the conditions tested in concurrence with the sponsor's conclusion.

Study title: STUDY TO EVALUATE THE POTENTIAL OF ACAMPROSATE TO INDUCE MICRONUCLEI ON THE POLYCHROMATIC ERYTHROCYTES OF CD-1 MICE

Key findings:

- Acamprosate negative for clastogenicity in the micronucleus test: no induction of micronuclei in polychromatic erythrocytes of bone marrow in mice at up to 2000 mg/kg on 2 consecutive days

Study no: 537/51
Study type: *In vivo*, clastogenicity
Volume # 19, and page #: 184
Conducting laboratory and location: [redacted]
Date of study initiation: April 7, 1993

GLP compliance: Yes
QA reports: yes (x) no ( )
Drug Acamprosate, **lot #** OTA 3049, **radiolabel** none, **and % purity:**[ ]%

Formulation/vehicle: Acamprosate in 1% (w/v) methyl cellulose

Methods:

Strains/species/cell line: CD-1 mice [ ]

Dose selection criteria:

Basis of dose selection: Toxicity study: see Range finding study below

Range finding studies: 3 mice/sex (ages 30-37 d, weights 20-22 g) received acamprosate in 1% (w/v) methyl cellulose at 2000 mg/kg PO (100 mg/ml, 20 ml/kg); no deaths or toxicity by acamprosate at 2000 mg/kg/d for 2 consecutive days; 2000 mg/kg considered acceptable upper limit (Topham *et al.*, UKEMS Sub-committee on Guidelines for Mutagenicity Testing. Report. Part I. Basic Test Battery. United Kingdom Environmental Mutagen Society, Swansea, pp. 119-141).

Test agent stability: No precipitate reported

Metabolic activation system: NA

Controls:

Vehicle: 1% (w/v) methyl cellulose

Negative controls: 1% (w/v) methyl cellulose

Positive controls: Cyclophosphamide (CPA.[ ] in physiological saline at 80 mg/kg (20 ml/kg)

Comments: Controls appropriate

Exposure conditions:

Incubation and sampling times: See under Study design below

Doses used in definitive study: 500, 1000, 2000 mg/kg (25, 50, 100 mg/ml, 20 ml/kg)

Study design: 10 mice/sex/dose received acamprosate orally on two consecutive days; positive controls used 5 mice/sex; acamprosate treated and negative control mice were sacrificed at 24 and 48 hours (5/sex/dose/timepoint) and positive control mice were sacrificed at 24 hours; femurs were isolated and ends removed, bone marrow was removed and bone marrow smears prepared and mounted on slides; slides were scored for relative proportions of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE), ratio of PCE/NCE and frequency of micronucleated PCE/1000; results from test and positive control mice were compared to those of the negative controls

Analysis:

No. of replicates: None

Counting method: PCE/NCE ratios and micronucleus frequencies/1000 cells; then counting of micronuclei in PCE up to 2000 PCE

Criteria for positive results:

1. Statistically significant increase in frequency of micronucleated PCE in at least 1 dose group in at least 1 time point
2. Significant increase in frequency of micronucleated PCE exceeding historical

vehicle control range

3. Evidence of increased (even if insignificant) frequencies of micronucleated PCE at other doses/time points, or dose response effect (even if insignificant)

Summary of individual study findings:

Study validity:

1. Acceptable variability between animals within group in the heterogeneity x^3 test
2. High dose (2000 mg/kg) acceptable as maximum dose for this assay
3. Controls were appropriate
4. Acceptable PCE:NCE ratio observed
5. Incidence of micronucleated PCE in vehicle control groups within historical control range
6. Minimum 8 animals/group evaluated
7. Significant increase in frequency of micronucleated PCE in positive control group

Study outcome:

There was no significant increase in the frequency of micronucleated PCE by acamprosate at any dose or timepoint (mean range 0.10-0.60) compared to controls (mean 0.25-0.45). The positive control CPA significantly increased the frequency of micronucleated PCE/1000 cells (26.43) compared to the control frequency ($p<0.001$). Thus, acamprosate tested negatively in the *in vivo* micronucleus test under the conditions tested in concurrence with the sponsor's conclusions.

Genetic toxicology summary: The following GLP *in vitro* and *in vivo* studies were conducted to evaluate the genotoxic and clastogenic potential of acamprosate.

Genotoxicity Studies with Acamprosate

Study	Test System	Dose Levels	Route	Study No.	Response
Reverse mutation	*S. typhimurium*	50-5000 µg/plate	*In vitro*	83058	Negative; appropriate tester strains were not used
Reverse mutation	*S. typhimurium* *E. coli*	8-5000 µg/plate	*In vitro*	537/50	Negative
Gene mutation	Chinese hamster V79 cells	100-3000 µg/mL	*In vitro*	85065	Increased number mutants at 300 mcg/ml in Test 1 and at 100, 1000, 3000 (but not 300) mcg/ml in Test 2 w/o metabolic activation. No increase in number of mutants in Test 3 at any ccn. w/o metabolic activation. No increase in number of mutants in any test, at any ccn. with metabolic activation.
Chromosomal	Human lymphocytes	10-300 µg/mL	*In vitro*	86002	Negative; dosing without

aberrations					metabolic activation was not up to limit
Micronucleus test	Mouse bone marrow	3000, 6000 mg/kg	PO	84008	Negative
Micronucleus test	Mouse bone marrow	500-2000 mg/kg	PO	537/51	Negative

Acamprosate was negative in two Ames tests, evaluating the mutagenic potential in the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, and in the *Escherichia coli* strains WP2 pKM101 and WP2 uvrApKM101, at concentrations up to 5000 mcg/plate with and without metabolic activation. Although study 83058 did not use the currently accepted types of strains, study 537/50 was a valid assay. Acamprosate was also negative for clastogenicity in the *in vitro* Chromosome Aberration assay in human lymphocytes at doses of up to 300 mcg/ml without S9 and 100 mcg/ml with S9, and in two *in vivo* Mouse Micronucleus Tests. Although statistical significance was observed in the increased number of gaps per cell with metabolic activation at 100 mcg/ml, the variation was not considered a genotoxic effect in the absence of other changes in structure and chromosome numbers, but did indicate a slightly toxic effect on the part of acamprosate. However, dosing in the Chromosome Aberration assay without metabolic activation and incubation time with metabolic activation did not achieve currently accepted criteria. The assay should be repeated.

Acamprosate produced equivocal results when tested for possible mutagenic activity at concentrations of 100, 300, 1000, and 3000 µg/ml (solubility limit) with and without metabolic activation (S9 mix) in the point mutation test at the HPRT locus in Chinese hamster V79 cells. A significant increase in the number of mutants was observed at a concentration of 300 µg/ml (5.9 times the number of spontaneous mutants) in the absence of metabolic activation in the first experiment and at concentrations of 100, 1000, and 3000 µg/ml (3.9, 4.9, and 5.3 times the number of spontaneous mutants, respectively) but not at 300 mcg/ml, without metabolic activation in the second experiment. A third test was conducted to confirm the results. In Experiment 3, conducted without metabolic activation, there was no significant increase in the number of mutants at any concentration. Also, when the results of all 3 tests were combined, no significant increases in the number of gene mutations were observed. However, because of the positive findings, the mutagenic or genotoxic potential for acamprosate cannot be ruled out. Although the solubility limit of acamprosate in Dulbecco H16 medium was stated as 3000 mcg/ml, no precipitate was described at this concentration, and the doses appear to be inadequate. Thus, this test should be repeated.

Genetic toxicology conclusions: Acamprosate was negative for mutagenicity in the Ames test, and for clastogenicity in the Chromosome aberration assay in human lymphocytes and in the in vivo Mouse Micronucleus test. Equivocal findings were observed in a point mutation assay using Chinese hamster V79 cells treated with 100-3000 mg/plate without metabolic activation; results were negative with metabolic activation. However, because of the positive findings, the genotoxic potential of acamprosate can not be ruled out. The highest concentrations used in the chromosome aberration assay and point mutation assay using Chinese hamster V79 cells and incubation times in the former assay with metabolic activation appear to be inadequate. Thus, the genotoxic potential of Acamprosate has not been fully evaluated. The *in vitro* chromosome abberration assay and point mutation assay using Chinese hamster V79 cells should be repeated using currently accepted dosing criteria and incubation times.

Labeling recommendations: It is recommended that the results of the genotoxicity studies be included in the product label as described under Genetic toxicology conclusions above. The label should be updated once the results of the requested studies have been submitted and reviewed.

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VI. CARCINOGENICITY:

Study title: **ACAMPROSATE: 91 WEEK ORAL (DIETARY ADMINISTRATION) CARCINOGENICITY STUDY IN THE MOUSE**

Key study findings:

- In mice given at oral doses of 25-400 mg/kg/day acamprosate for 91 weeks, there was no significant increase in treatment-related neoplasms.
- The study was terminated at 91 weeks due to high animal mortality in all groups.
- The study is unacceptable due to inadequate dosing, nematode infestation, and histopathologic assessment of low and mid-dose groups that was inadequate for conducting a trend test for tumor incidence.

Study number: 6894-537/27
Volume # 20-24
Conducting laboratory and location: [redacted]
Date of study initiation: 28 July, 1989
GLP compliance: Yes
QA report: yes (x) no ( )
Drug lot # OTA 3011, [redacted] purity
CAC concurrence: No

Study Type: 2 year bioassay
Species/strain: CD-1(ICR)BR [redacted]
weights 22.5-34.6 g males, 17.6-28.1 g females
Number/sex/group; age at start of study: 51/sex/group; ages approximately 28 days
Animal housing: Caged in groups of 3 in [redacted] cages with stainless steel mesh floors suspended over cardboard-lined trays, in a single exclusive room, air-conditioned to provide a minimum of 15 air changes/hour and routinely maintained at a temperature to 19-25 °C and a relative humidity of 40-70%, with light cycle of 12 hours light and 12 hours darkness.
Formulation/vehicle: Test article as a white powder for admixture with the diet/vehicle was powdered diet
Drug stability/homogeneity: Analysis of test diet: samples from top, middle, and bottom taken prior to and during study (weeks 1, 10, 23, 36, 49, 62, 75, and 88) for stability, homogeneity and achieved concentration. Formulations demonstrated homogeneously mixed and stable for period of use. Certificates of Analysis provided.

Methods:

Doses: 0, 25, 100 and 400 mg/kg/day

Mean Compound Consumption During Weeks 1-88 (mg/kg/d)

	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d
Males	24.8	99.2	398.4

Females	24.8	99.3	397.7

Basis of dose selection: The sponsor did not provide a rationale for dose selection. In a 13-week study of subacute toxicity study in mice (Report No.[ ] .138/88827, see Appendix 3), toxic effects of dietary acamprosate administration at doses up to 2000 mg/kg/d were increased water consumption at 1500-2000 mg/kg/d in males and females, increased plasma calcium at 1000, 1500 and 2000 mg/kg/d in males, increased urinary calcium excretion at 500, 1000, 1500 and 2000 mg/kg/d in males and females, slightly decreased liver weights at 1000, 1500 and 2000 mg/kg/d in males, and decreased heart weights at 2000 mg/kg/d in females. An MTD was not identified; the MTD is considered to be greater than the highest dose tested (>2000 mg/kg/day). The high dose selected for the 91-week carcinogenicity study in mice was 400 mg/kg/d.

Restriction paradigm for dietary restriction studies: Rat and Mouse Maintenance Diet No. 1, expanded, ground fine [ ] *ad libitum*, fasted overnight before necropsy. Drinking water filtered tap, changed daily, *ad libitum*.

Route of administration: Oral by admixture in the diet

Frequency of drug administration: Continuous in diet

Dual controls employed: Yes; both negative controls were untreated powdered diet.

Interim sacrifices: No

Satellite PK or special study group(s): No

Deviations from original study protocol: Actual temperature range 14-28degC on several occasions. Humidity deviated from specified range of 40-80% on a few occasions to 82% and 36%. Female animals were deprived of water overnight during one day in week 14 due to improperly connected automatic watering system. Sponsor address changed during study.

Statistical methods: Sponsor:

ANOVA, t-test: Body weights week 0

ANOVA, Regression and Dunnett's: Body weight gain, mean food consumption, Hematology.

Kaplan-Meier technique: Survival probability

Log-rank procedure: Survival curves

Two sided heterogeneity chi-squared: Comparison of two control groups

Tumor analysis: One-sided tests for increasing and decreasing incidence with increased dose between control groups and group 4. Non-fatal tumors analyzed using fixed intervals 0-50 weeks, 51-80 weeks, 81-90 weeks (males only), 81-91 weeks (females only, and terminal kill). Permutation tests to establish significance of findings wherever fatal or non-fatal tumors observed with total incidence of at least 3 but less than 10, or whenever total combined incidence was less than 10 but at least 3. Then exact permutational sampling distributions of test statistics obtained. Fatal and non-fatal results combined in accordance with IARC annex, and reported only if significant.

Agency Statistical Reviewer: statistical test on the survival data to compare the survival curves of the four dosage groups. Then, this reviewer performed two statistical tests on the tumor incidence. The first was a trend test intending to identify any significant positive dose-response linear trend for the tumor incidence. The second was a pairwise test intending to identify any significant difference between the high dose and

placebo groups. Two control groups were combined in both tests. They were stratified on properly divided time intervals to adjust for intermittent mortality. For the mouse study, time intervals were 0-40, 41-70, 71-89, and 90-93 (terminal sacrifices) weeks.

This reviewer followed FDA routine procedures to perform the statistical tests in this carcinogenicity review. Rare tumors are defined as those with incidence rate of 1% or less in historical control data or concurrent control groups, otherwise it is a common tumor. In the trend test, significance level is set to be 0.025 for rare tumors and 0.005 for common tumors. In the pairwise comparison between high dose (400 mg/kg/day) and placebo, significant levels are 0.05 for rare tumors and 0.01 for common tumors. These adjustments for multiplicity are believed to control the overall type I error at around the 10% level in a standard two-species-two-sex study.

Observations and times:

Clinical signs: Daily
Body weights: Baseline, daily, weekly for 16 weeks, then every 4 weeks
Food consumption: Baseline, daily, weekly for 16 weeks, then every 4 weeks
Hematology: At necropsy: white blood cell count only
Clinical chemistry: Not done.
Organ weights: Not done.
Gross pathology: At necropsy
Histopathology: at necropsy

Histopathology Inventory*

	Control 1	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d	Control 2
Abdominal Cavity					
Adrenals	X	X	X	X	X
Aorta					
Bone Marrow Smear	X	X	X	X	X
Bone	X	X	X	X	X
Brain	X	X	X	X	X
Cecum	X	X	X	X	X
Cervix					
Clitoral Gland					
Colon	X	X	X	X	X
Connective Tissue					
Cranial Cavity					
Duodenum	X	X	X	X	X
Ear					
Epididymis	X	X	X	X	X
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian Tube					
Foot/Leg					
Gall Bladder	X	X	X	X	X
Gross Lesions	X	X	X	X	X
Harderian Gland					
Heart	X	X	X	X	X
Hypophysis					
Ileum	X	X	X	X	X

Jejunum	X	X	X	X	X
Kidneys	X	X	X	X	X
Lachrymal Gland					
Larynx					
Liver	X	X	X	X	X
Lungs	X	X	X	X	X
Lymph Nodes, Cervical					
Lymph Nodes, Mandibular	X	X	X	X	X
Lymph Nodes, Mesenteric	X	X	X	X	X
Mammary Gland	X	X	X	X	X
Muscle (skeletal, quadriceps)	X	X	X	X	X
Nasal Cavity					
Optic Nerves					
Oral Cavity					
Ovaries	X	X	X	X	X
Pancreas	X	X	X	X	X
Parathyroid	X	X	X	X	X
Penis					
Peripheral Nerve					
Pharynx					
Pituitary	X	X	X	X	X
Prostate	X	X	X	X	X
Rectum	X	X	X	X	X
Salivary Gland	X	X	X	X	X
Sciatic Nerve	X	X	X	X	X
Seminal Vesicles					
Skin	X	X	X	X	X
Spinal Cord	X	X	X	X	X
Spleen	X	X	X	X	X
Sternum	X	X	X	X	X
Stomach	X	X	X	X	X
Tail					
Testes	X	X	X	X	X
Thoracic Cavity					
Thymus	X	X	X	X	X
Thyroid	X	X	X	X	X
Tongue					
Trachea	X	X	X	X	X
Ureter					
Urinary Bladder	X	X	X	X	X
Uterus	X	X	X	X	X
Vagina					
Zymbal Gland					

*X: Organs examined in all mice that died and were sacrificed.

Toxicokinetics: Blood samples taken in weeks 90 (males) and 91 (females).

Results:

Mortality:

End of Study Survival Rate

Acamprosate Dose (mg/kg/d)

	Control 1	25	100	400	Control 2
Males	59%	49%	41%	55%	65%
Females	55%	61%	55%	73%	67%

FIGURE 1

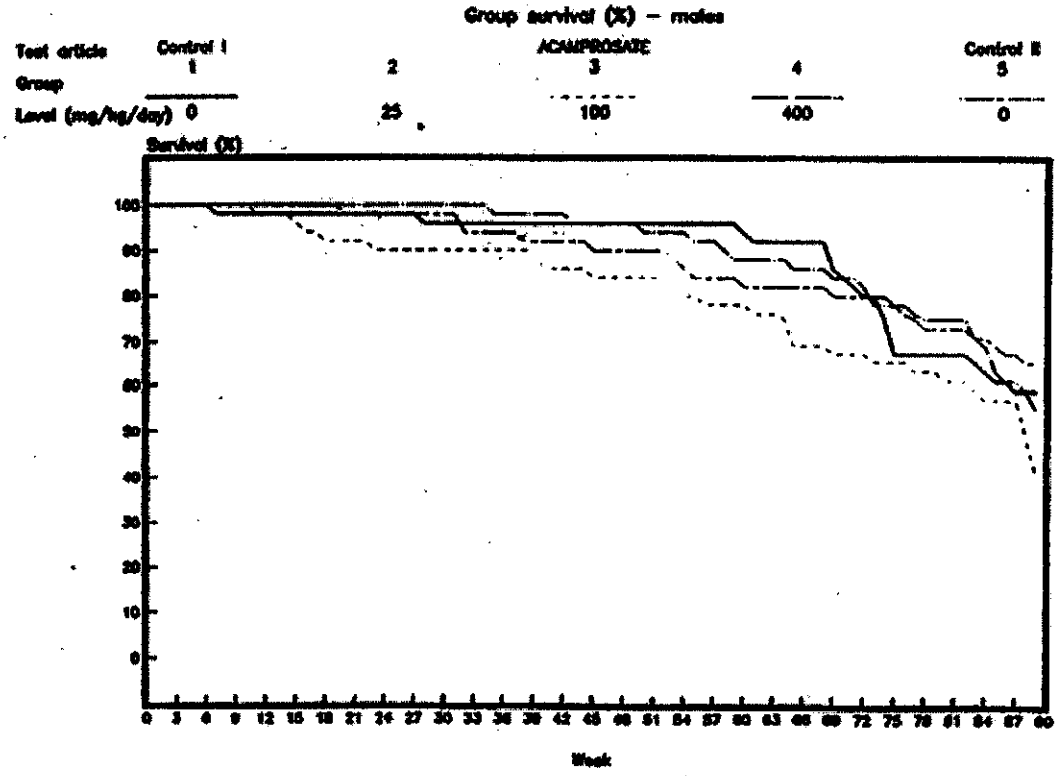
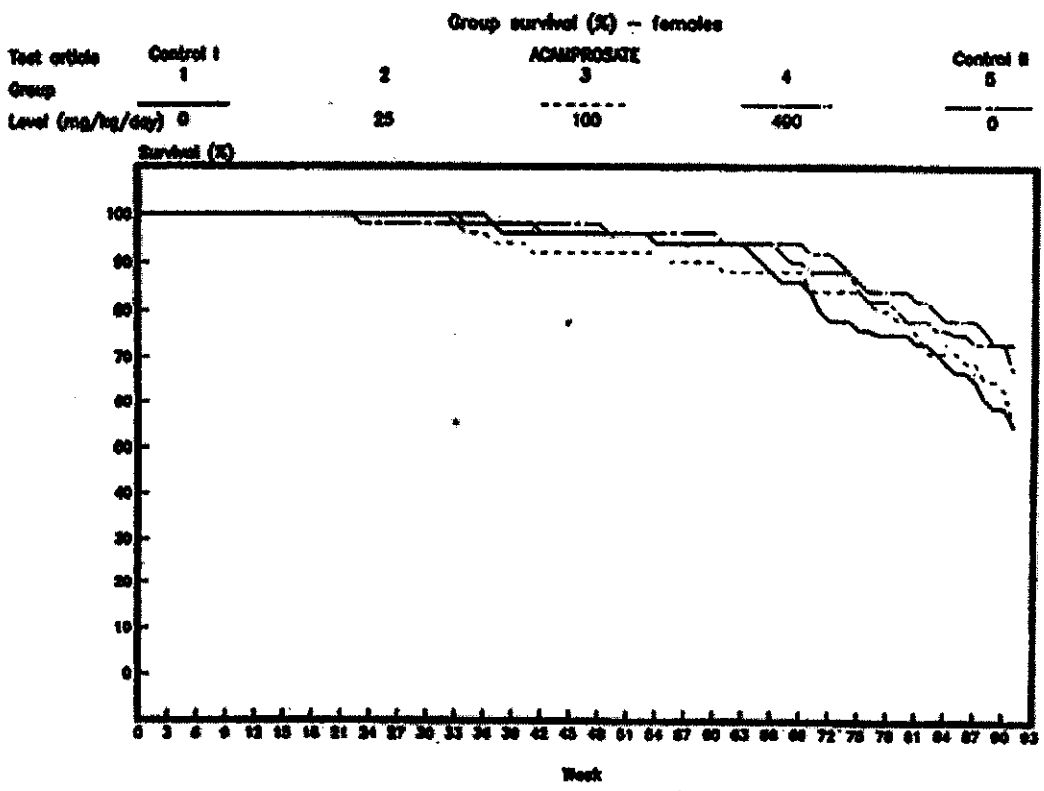


FIGURE 2



Cause of Death (#Animals)

	Acamprosate Dose (mg/kg/d, Males)					Acamprosate Dose (mg/kg/d, Females)				
	C1	25	100	400	C2	C1	25	100	400	C2
Total deaths	22	26	31	23	22	23	20	23	14	18
Procedure/Trauma	0	0	0	0	2	1	0	1	0	0
Urogenital tract lesion	5	10	14	3	7	0	0	0	0	1
Skin/appendage lesion	0	2	2	4	2	0	1	0	0	0
Eye lesion	0	1	0	0	0	0	0	0	0	0
Neurological lesion	0	0	0	1	0	0	0	0	0	0
Cardiovascular lesion	0	1	0	0	1	1	2	0	0	0
Respiratory lesion	0	0	0	0	1	0	1	0	0	0
Foot lesion	0	0	1	0	0	0	0	0	0	0
Gastrointestinal lesion	0	0	0	0	1	0	0	0	0	0
Uterine lesion	0	0	0	0	0	3	0	1	1	2
Ovarian lesion	0	0	0	0	0	2	3	1	2	2
Other lesion	2	0	1	0	0	0	0	0	0	0
Glomerulonephropathy	0	1	1	1	1	2	1	1	1	3
Amyloidosis	3	1	1	7	4	1	1	0	0	0
Hemolymorphoreticular tumor	4	3	2	2	1	6	4	4	1	3

Skin Subcutis tumor	3	1	3	3	0	2	1	6	0	0
Lung tumor	0	0	1	1	0	0	0	1	2	1
Liver tumor	2	2	2	0	0	0	0	0	0	0
Mammary tumor	0	0	0	0	0	3	1	3	1	4
Uterine tumor	0	0	0	0	0	0	1	1	2	2
Histiocytic Sarcoma	1	0	0	0	0	1	1	0	1	0
Other tumor	1	1	1	0	1	1	1	0	0	0
Multifactorial	0	1	0	0	0	0	1	1	0	0
Undetermined	1	2	2	1	1	0	1	3	3	0

Clinical signs: No treatment-related clinical signs.
Body weights: No treatment-related effects.

Mouse mean body weight at 1 year

Dose (mg/kg/day)	Males (g)	Females (g)
0 (Control 1)	43.8	35.2
0 (Control 2)	42.8	34.4
25	45.1	35.5
100	44.1	35.9
400	44.8	35.2

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FIGURE 3

Group mean body weight (g) – males